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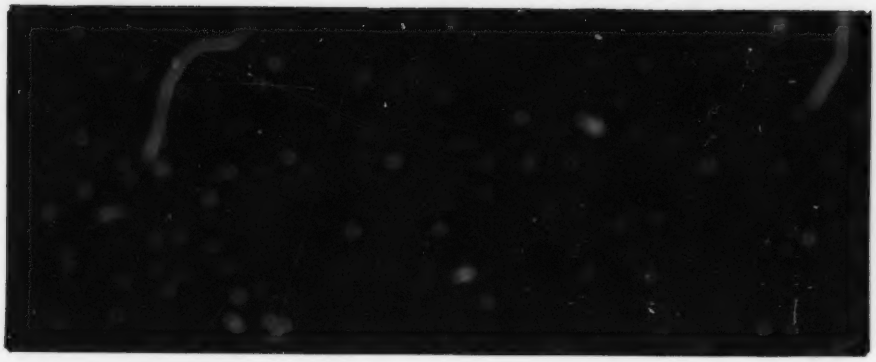

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Biology and Biological Control Potential of a Wheat Midge Parasite, *Pirene penetrans* in Saskatchewan

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Executive Summary

ABSTRACT

The life stages, biology, behaviour and potential of a parasitic wasp Pirene penetrans as a biological control agent of wheat midge in Saskatchevan was determined. For identification purposes, the egg, larva, prepupa, pupa and adult stages were described and illustrated. The development of parasites in larvae within wheat heads, and later in midge larvae from the soil was followed throughout the growing season.

The general activity, egg-laying behaviour and number of eggs produced by parasite females were determined by field and laboratory studies. The percent parasitism and impact of the parasite on midge populations were determined.

SUMMARY

A parasitic wasp, Pirene penetrans was observed attacking the wheat midge in 1984 in northeastern Saskatchewan. Because it was killing up to 50 percent of the midge in some populations, further study of its life stages, biology, behavior and impact on midge populations in Saskatchewan were undertaken. Such knowledge is necessary to clearly separate the immature stages of this parasite from other parasite species that might occur before any introductions of additional parasitic species from Europe could be considered.

The parasite has five life stages: adult wasp, egg, larva, prepupa and pupa. The adults begin emerging from the soil in early July. Mating was not seen, either in the laboratory or the field. The parasite lays its eggs in the eggs of the midge. Parasite eggs take 4-5 days to develop and begin hatching at about the same time that the midge eggs hatch. The tiny parasite larva grows in the midge larvae while the midge larvae is feeding and maturing on the wheat kernels. The midge larvae drop from the wheat head during August and early September. The first stage parasite larva (within the host midge larva) reaches full size by late August and early September and then changes to a second stage larva to overwinter within its host. In late April or early May development of the parasite resumes. About mid-June it changes to a prepupa after its host midge larva has spun a pupal cocoon. The prepupa is distinctive in shape and appearance from the preceding parasite larval stage and the succeeding pupal stage. The pupa gradually transforms to the adult stage within the midge pupal cocoon. Emergence of both the midge and its parasite began in early ~~June~~ July in 1987 and continued until August 6.

The percent parasitism of midge larvae taken from soil samples at weekly intervals increased from 12% in early May to 25% by mid-October. The percent parasitism of the midge larvae in wheat rose to over 50% before harvest.

Activity studies in the field showed that more midge adults were trapped close to the soil surface in the wheat canopy while parasite adults were trapped in the upper canopy. Also midge adults were trapped in the evening while

parasite adults were trapped more in mid-day and more during sunny than cloudy weather. Egg-laying by parasites into midge eggs on wheat heads was also greater in sunny than cloudy weather.

Each female parasite has the potential of laying about 200 eggs on average. This means that the parasite could under optimum conditions destroy up to 200 midge larvae before they could mature to the adult midge stage.

Autumn surveys in midge-infested rural municipalities in the northern and eastern regions of the midge-infested area, determined the numbers of midge larvae/m^2 and the numbers of parasitized midge larvae/m^2. The soil population of midge dropped by about 37% from 1986 to 1987. The percent parasitism of wheat midge by the wasp, was 31 and 23% respectively for 31 rural municipalities sampled in 1986 and 52 in 1987. Analysis showed a direct relation between the number of wheat midge attacked by the wasp and the total midge density. As the numbers of midge increase more are attacked. This "density-dependent" response is considered an essential attribute for an efficient natural control agent. The parasite wasp is therefore considered to be a major factor in the natural control of wheat midge in the province.

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Technical Report

Biology and Biological Control Potential of a Wheat Midge Parasite, *Pirene penetrans* in Saskatchevan

ABSTRACT

The life history, biology, behavior and potential of Pirene penetrans Kirby for controlling wheat midge, Sitodiplosis mosellana Gehin in Saskatchevan was investigated in 1986 and 1987.

The stages of P. penetrans are the egg, two larval instars, a prepupa, a pupa and the adult. Adults are shiny black; the male has prominent rust-coloured eyes. In 1987, the female parasites emerged over a period of about a month, from early July to early August. Mating was not observed either in the field or laboratory. The parasite lays eggs in the eggs of the midge. Superparasitism with 2-4 eggs per midge egg, was frequently observed in the field but not the laboratory. Parasite eggs begin to hatch about 4-5 days after being laid, at about the same time the host midge egg hatches. In the field, midge eggs containing parasite eggs were found from July 2 to August 6.

A frequency distribution of measurements of 693 parasite larvae dissected from midge larvae showed two distinct larval instars. The first instar develops in the midge larva while the midge larvae is feeding upon the wheat kernel. The first instar reaches full size in late August or early September, then usually changes to the second instar to overwinter along with its host. The parasite larva resumes development in late April or early May as the soil temperature rises, until it reaches full growth in early June. About mid-June it changes to a prepupa that is distinctive in appearance from the preceding larval stage and the succeeding pupal stage. The pupa gradually transforms to the adult stage within the midge pupal cocoon. Emergence of the adult midge and parasite began in early July and continued to early August.

The percent parasitism of midge larvae dissected from midge larvae from soil samples increased from 12% in early May to 25% by mid-October. Mortality of both the midge and parasites was observed in June. Mortality of parasites within live hosts also occurred. The percent parasitism was followed in the midge eggs and developing larvae in wheat heads. The unparasitized larvae dropped from the wheat heads more rapidly than the unparasitized larvae.

Activity studies in the field were made with "sticky board" traps and direct observation. Midge adults were trapped close to the soil surface mostly in the evening. Parasite adults were trapped mostly in the upper canopy during mid-day. More activity of the parasites occurred during a sunny, warm day than during a cloudy, rainy day. Oviposition activity was higher during sunny than cloudy weather.

Female parasites laid into midge eggs in the laboratory in small cages. An average of 12 parasite eggs were laid in a 2 h period. Females lived from 1-16 days; males no longer than 8 days. Parasite egg-laying behavior entailed grooming, abdomen flexing, searching, probing and finally egg-laying.

Each female parasite produces up to 200 eggs and under optimum conditions could presumably destroy this number of midge. Dissections of field-collected females showed that they had laid an estimated 53% of their eggs when collected.

Autumn soil surveys of 31 rural municipalities (RM's) in 1986 and 52 RM's in 1987 showed that midge numbers in soil dropped from 642 larval cocoons/m^2 to 394/m^2 from one year to the next. In both years percent parasitism varied from 0 to over 80%. Regression analysis showed that a density dependent relationship existed between the number of midge larvae parasitized and overall midge larval density. P. penetrans was the only parasite found and is considered to be playing a major role in reducing overall midge density.

Biology and Biological Control Potential of a Wheat Midge Parasite, Pirene penetrans in Saskatchewan

Background

The wheat midge outbreak in Saskatchewan, first reported in 1983, and the damage it caused (Olfert et al, 1985) necessitated development of a program that would include cultural, chemical and biological control as well as pest monitoring. The obvious components of biological control worthy of investigation were predation and parasitism. The role of carabid predators and effects of insecticides on these predators has been investigated (Floate 1987). A parasitic wasp, Pirene penetrans (Kirby) was first observed in July 1984 when it emerged along with the wheat midge. This parasite had been found attacking wheat midge in Manitoba during the outbreak that took place in the 1950's. The spread of the parasite in northeastern Saskatchewan appeared to follow the spread of the midge. By autumn 1968, parasitism in five study plots situated at intervals between Aylsham and Gronlid, averaged 50% (Doane unpublished data).

Therefore, the parasite appeared to have high potential as a natural biological control agent for wheat midge. Its biology and potential for control have never been studied in detail. This was necessary before any possible introductions of additional parasitic species from Europe could be considered.

Objectives

The objectives of this research were to clearly identify the immature stages of the parasite in its wheat midge host, to determine its biology, behavior, developmental period, distribution and potential as a biocontrol agent in Saskatchewan.

Experimental Methods

1.0 Morphology and description of stages of Pirene penetrans

Measurements of stages were obtained with an ocular micrometer and a compound microscope. The various stages were illustrated and/or photographed.

1.1 The adult

The length of 50 males and 32 female P. penetrans adults that emerged in the laboratory was determined. Numbers of males and females emerging in the laboratory were counted over a period of two months and the sex ratio determined. Sex ratios were also obtained from emergence cages and on sticky board traps in the field.

1.2 The egg

The length and width of 25 unlaid eggs dissected from the oviducts of each of five females were obtained. The dimensions of 28 laid eggs were obtained in a similar fashion after they were dissected from the host midge eggs.

1.3 The larva

Morphological studies were conducted on the larvae to identify the instars and to enable separation of the stages in relation to phenological growth.

In 1986, overwintering midge larvae were collected from the field in September. Larvae were removed from the cocoons by soaking them for about 30 seconds in an 8% aqueous solution of potassium hydroxide. Some were dissected immediately and any parasite larvae found were measured. The remaining cocoons were stored in soil at $2^{o}C$ for two months or more in order to break diapause. Cocooned larvae were then brought out of storage at intervals and alloved to develop before being dissected to obtain parasite larvae, prepupae and pupa, which were measured and/or illustrated or photographed. The head capsule width

(HCW), head capsule length (HCL) and body length (BL) were obtained for 145 larvae at all stages of development. These measurements were subjected to cluster analysis and later, the frequency distribution determined and the cumulative percentage plotted to show the "point of inflexion" that indicates change from one instar to the next.

In 1987, a plot was set up on the Bodnaryk farm (RM273, NE-16-28-1) near Veregin and divided into 16 equally sized subplots, 10 by 10 m. Five soil cores, each 2.54 cm diameter by 15 cm deep were taken at random from each subplot weekly from early May until mid-October. The samples were stored at 2°C until they were processed by wet sieving (Doane et al 1987) to remove wheat midge cocoons, larvae or pupae. These were dissected and the HCW, HCL and BL obtained on a variable number of P. penetrans larvae each week.

In July, when the wheat had begun flowering, two plants were removed from each subplot at weekly intervals during the first and last of the midge egg-laying period and at intervals of 3-4 days during the peak egg-laying period. Midge eggs and larvae when present were removed from the heads and dissected. (See Section 2.5). Samples of parasite larvae ranging from 5 to 30 (depending upon their availability) were measured each week until September 9, when the plot was harvested and midge larvae were no longer available. For analysis, measurements from soil data and wheat head data were combined to give a total of 693 measurements for all three parameters. Data was analyzed by determining the frequency distribution of HCW.

Observations were also made on many larvae to determine whether mouth parts were present in the different instars and to establish other morphological characteristics that could be used to separate the stages. The different stages and morphological features were described in sketches and photographs.

In an attempt to show the presence or absence of mouth parts, larval specimens of different age and development were fixed and sectioned. For sectioning, early second and late second instar P. penetrans larvae were fixed overnight in buffered glutaraldehyde at 0°C (ph 8.2), post-fixed in O_sO_4 for 1 h

at room temperature, dehydrated in a graded alcohol series and baked for 48 h at 60°C (Weakly 1981). One µm sections were cut with a LKB Ultratome III ultramicrotome and subsequently stained with toluidine blue before making permanent mounts.

Whole mounts were also examined for mouth parts. In one procedure, larvae considered to be in the early and late second instar were fixed in Bouin's fixture (Baker 1958) overnight and then stained with Rhodamine B (in 50% ethyl alcohol) for 4 h during dehydration in a graded alcohol series. The dehydrated and stained larvae were mounted in permount under a cover slip. In another procedure, semi-permanent slides were made of third instar (or late second instar) larvae that were cleared and mounted in de Faure's solution under a cover slip (Beirne, 1955).

1.4 Prepupa and Pupa

Only a few prepupa were measured in 1986 and illustrated. Pupa were photographed but not measured.

2.0 Seasonal development and percent parasitism

2.1 Spring Survey - 1987

The Saskatchevan Agriculture wheat head survey of August 1986, showed that the highest midge populations were in an area south of Veregin (RM237) and northeast of Yorkton (RM243). On May 4, a survey was made of 17 fields in that area to locate fields suitable for biological studies, to determine midge cocoon densities, percent parasitism and stage of parasite development. Ten cores were taken from each of the 17 fields and the larval cocoons extracted by wet sieving in the laboratory. The larvae were removed from the cocoons using an 8% aqueous solution of potassium hydroxide which dissolves the cocoon casing. Midge larvae were then dissected as before and measurements of HCW, HCL and BL obtained. The stage of development of parasite larvae was also estimated.

2.2 Seasonal development of P. penetrans and percent parasitism of soil-collected midge larvae

The sixteen subplots at the Bodnaryk site were sampled from May 4 to October 14 and samples extracted as described under section 3.1. Larvae from cocoons were dissected throughout the season and the percent parasitism determined. The stage of instar development was estimated from their size and appearance (see Section 3.1).

2.3 Soil depth at which parasitized and unparasitized midge were found.

Two soil samples, 10-16 by 15-24 cm (4x6 in) were taken from the 16 subplots at the Bodnaryk site on July 2. Each sample was composed of four successive layers 2.54 cm (1 in.) in depth. Each subsample within a sample was placed in a separate plastic bag stored at $2^{o}C$ and later extracted by salt flotation (Doane et al 1987). Any midge larvae in cocoons, free larvae and midge pupal cocoons were dissected to determine parasitism.

2.4 Emergence of midge and parasites in the field

Emergence cages used in an earlier study (Doane unpublished) were used to follow the seasonal emergence. Two traps were placed in each of the 16 subplots at the Bodnaryk site on June 25. Strips of plastic coated with Tanglefoot and placed within the cages trapped the emerging midge and parasites. These strips were changed every week until August 6.

2.5 Parasitism in the field in relation to location of midge egg in florets

At the Bodnaryk site, two wheat plants were taken at random from eight even numbered subplots at intervals of a week or less from July 2, when some of the primary heads were in the boot stage and early flowering stage, until harvest on September 9. The growth stage (Zadok et al 1975) was estimated for primary heads and tillers on each plant. The spikelets per head were counted from the base to the tip. The florets within each spikelet were dissected and the number of midge

eggs counted on the inside and outside of the glume, inside and outside of lemma, the palea and the rachis. Eggs found at each location were dissected to determine the percent parasitism. During the examination, both live and dead eggs, parasitized and unparasitized were recorded. Therefore, the influence of location of the eggs to accessibility of P. penetrans, eventual parasitism and mortality was examined in this manner. Later the numbers of larvae found in each grain per floret, spikelet and head were determined in a like manner. Larvae were also dissected to determine the percent parasitism.

3.0 Seasonal and diurnal activity of P. penetrans and midge adults in the field.

3.1 Seasonal captures on "sticky board" traps

A 40 x 40 m plot was laid out on Glutes farm (RM273 NW-11-28-1) and divided into 16 subplots, each 10 by 10 meters. The plot was seeded to spring wheat Neepava (May 15). On July 3, two boards 20 x 30 cm were mounted on posts in the same direction so that the longitudinal axis of each board was centered along each stake, with the bottom edge of the lower board 10 cm above the soil surface and the bottom edge of the upper board 5 cm above the lower board. Traps were placed in each of the 16 subplots, so that the boards on each of four traps faced each cardinal direction, a sheet of 4 mil plastic also 20 x 30 cm, was stapled to each board and covered with a thin layer of Tanglefoot using a paint roller. Sheets were removed and replaced by fresh plastic and Tanglefoot at approximately weekly intervals. When a sheet was removed it was placed sticky side down on stiff cardboard and divided into quarters. They were stored at 7°C until the numbers of parasites and hosts could be determined under a stereo microscope.

3.2 Diurnal activity

These experiments were conducted to: determine the daily flight activity of P. penetrans adults in relation to wheat midge adults; determine daily patterns of activities of P. penetrans females on wheat heads and to observe when egg-laying occurs.

For the first experiment "sticky board" traps were placed in eight of the odd numbered plots at the Glute site so that two traps faced each of the cardinal directions. The traps and methods used were as described under 3.1. Plastic sheets were placed on boards at 6:00 hrs on July 22 and July 23 and coated with Tanglefoot. These were changed every four hours until 22:00 hrs. As before, sheets of plastic with the Tanglefoot-trapped insects were placed on sheets of cardboard and stored at 7°C until they could be examined under a stereo microscope.

For the second experiment, observations were made every two hours (July 22 and 23) on the number of parasites and on their searching and ovipositional behavior for ten wheat heads randomly chosen from both the lower (wheat heads 55-66 cm tall) and upper canopy (wheat heads 86-96 cm tall) in each of the 16 subplots. Females that were inserting their ovipositors between the glumes were recorded as ovipositing and could be distinguished from females that were "resting" or "searching".

Environmental parameters measured every two hours beginning at 6:00 hrs and continuing until dusk (about 20:00 hrs) were: air temperature (°C), relative humidity (in %), illumination (in lux) and wind speed (Kph). These records were obtained for July 22, a cloudy day with intermittent showers and for July 23, a mostly sunny day with broken cloud cover.

Differences in numbers of midge and parasites trapped on sticky board traps were compared for the following main effects using chi square analysis: date, time of day, sex, upper and lower boards and direction. Interaction of these effects also were considered. Chi square analyses also were used to analyze the activity on wheat heads where the main effects were date, time of day, and upper and lower canopy along with interaction.

4.0 Behavior and development studies in the laboratory

4.1 Rearing of wheat midge

Containers of soil containing wheat midge cocoons (ca. 125 cocoons/500 gr sort) that had overwintered in the field were placed in an environment room at 23°C and 65 ± 1% relative humidity. Adult midge emerging about 23 days later from the soil aggregated in clear plastic cones placed over the containers (Hinks and Doane 1988). When sufficient numbers had emerged, such as 20-100 every second and third day they were transferred to a larger cage (27x28x30cm) for oviposition (Fig. 1). The midge were fed with a 20% sucrose/honey solution diffusing into a wick from small vials which were placed on narrow shelves in the upper third of the cage.

Wheat heads, with stems 15-25 cm long and at the appropriate stage of development (emerging from the boot to early flowering) were collected from Neepawa wheat grown in a growth chamber. The heads were then placed in Hoagland's nutrient solution and then into the rearing cage. The wheat head (or sometimes two heads for a large number of midge) was placed in the cage at about 13:00 h so that the midge would oviposit in the late afternoon and evening. The light in the environmental room began to diminish by 19:00 hrs and was off by 22:00 h. The following morning the head was removed from the cage at about 8:00 h and examined for eggs.

4.2 Rearing of parasites

Adult parasites emerging from the midge-infested soil were aspirated into a vial and transferred to small cages (emergents from one date in the same cage). The cages were inverted round containers of clear plastic (12 cm diameter by 12 cm high) with tight fitting lids (Fig. 2). The lid became the floor of the cage and was lined with moist filter paper. A hole, two cm in diameter, was drilled into the roof of the cage and closed with fine nylon mesh to allow for ventilation. A second hole, the same diameter, made in the side of the cage and plugged with a cork provided an easy access. Feeding wicks saturated with 20%

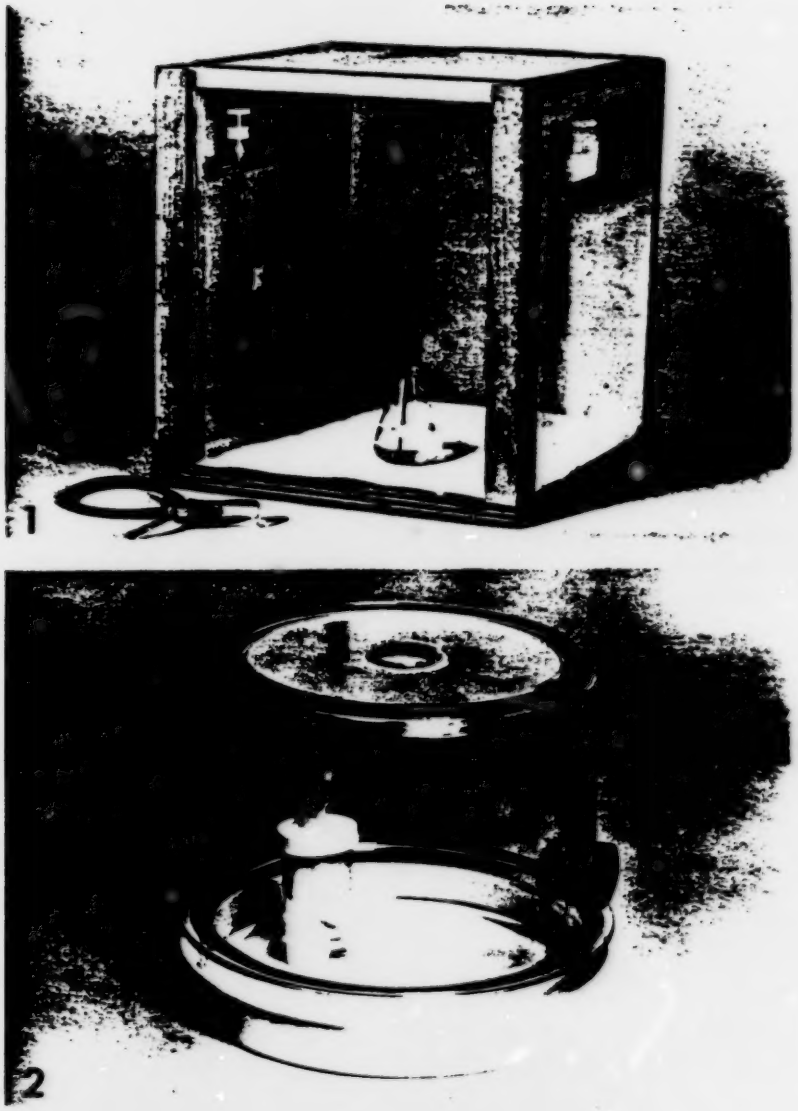

Fig. 1 and 2. Cages for rearing midge (1) and parasite oviposition (2)

sucrose/honey solution were provided as a food source. The adults were contained in the cages during oviposition and behavior studies until they died, at which time they were dissected for egg counts.

4.3 Parasite development

Wheat heads found to have midge eggs were used immediately in experiments on parasite development (at 23°C). The stems of the heads were either cut short and the heads inserted into flat-resting clamps so that the heads could be placed erect inside the parasite cages, or the stems were left intact. In the latter case, a small hole was made in the base of a parasite cage and the stem slipped through so that the head sat erect within the cage. The stem then was placed in a flask of Hoagland's solution situated beneath the cage. A single female parasite (two or more days old) was placed on a wheat head with midge eggs and alloved access to the head for two hours before being removed.

Heads with short stems were then placed in moist petri dishes for short periods of time (2-5 days at 23°C) before examination, and those with longer stems were left in Hoagland's solution at 23°C for longer periods of time (7 plus days) before examination. It was found that midge survival was better in heads kept under high moisture conditions, so heads in Hoagland's solution also were placed in a small, closed, moist container within the environment room (a glass dessicator jar).

Heads were prepared as above whenever adult midge and parasites were available and laying. Those heads successfully exposed to ovipositing midge and parasites were sacrificed at intervals of one or two days initially, and then at weekly intervals once the parasite eggs had hatched. A minimum of three replicate heads were examined per time interval. During examination of the heads, midge eggs or larvae were removed from the heads, counted, and dissected to check for parasitism. The number of parasites and their stage of development were noted and, parasite larvae, if present, were measured.

4.4 Parasite oviposition behavior

In three instances, wheat heads with midge eggs were used in detailed behavior studies within the laboratory. Each head used was placed in the parasite rearing cage with 1-2 female parasites and activity was closely monitored and recorded for 2-3 hours.

In one of these studies the wheat head was initially mapped so that the spikelets were numbered and the location of midge eggs recorded before placing the head with a female parasite. During her searches on the head, the spikelet number being visited was recorded, and periods of oviposition were timed. After the female was removed, the wheat head was dissected and the total number of midge eggs and number of parasitized eggs were recorded for each location within each spikelet. From this information, the estimated average time for one oviposition event was calculated. Three females in the process of searching and ovipositing also were photographed.

Lastly, a wheat head with midge eggs was left for six days at 23°C by which time the eggs had hatched. The first instar midge larvae (0.429 mm $\pm$ 0.053 long; n=5) were then removed from the head and approximately 35 were placed on the surface of a new clean wheat head. The wheat head was exposed to a female parasite for two hours before retrieving the midge larvae for dissection. The experiment was repeated two more times. In these instances 50 midge larvae (six days after midge oviposition) were placed on each of two heads and each head was exposed to a female parasite for two hours.

5.0 Reproductive potential of P. penetrans

In 1986, 7 females that had emerged in the laboratory and never laid eggs were dissected and the numbers of immature and mature eggs in the oviducts counted. Field collected females (n=10) were also dissected to determine their complement of eggs.

In 1987, female parasites that emerged in the laboratory were dissected to determine their numbers of eggs. Dissections were made on females of various ages that had and had not laid eggs.

6.0 Autumn soil survey for wheat midge larvae in cocoons and percent parasitism

Soil surveys were conducted in autumn 1986 and 1987, September 15 to October 15. Ten soil cores were taken in four fields from 31 rural municipalities (RM's) in 1986 and 52 RM's in 1987. Cores from each field were bulked and stored at 2°C until the soil was processed by salt flotation (Doane et al 1987). Cocoons so obtained were counted under a stereoscope, and then the larvae dissected to determine the percent parasitism. Midge density and percent parasitism was calculated for each RM. The mean density of midge per metre squared and the percent parasitism for RM's within each of nine Agricultural Representative Districts (ARD) in 1986 and 13 in 1987 was also obtained. Also regression analysis was used to examine the relationship between total midge larvae density and density of parasitized larvae. The effects of individual RM's and A.R. districts was examined by covariance analysis.

Results and Discussion

1.0 Morphology and description of stages of Pirene penetrans

1.1 The adult

The adult of Pirene penetrans was first described by Kirby (1800) as Ichneumon penetrans and by Johnson (1936) as Macroglenes penetrans Kirby, who gave the synonomy up to that time. More recently, the taxonomic position has been given by Graham (1969) who included penetrans under the genus Pirene, family Pteromalidae.

Both sexes are shiny black; the wings ar iridescent in sunlight. The males have prominent rust-coloured eyes while the females (Fig. 3) have less prominent dark brownish eyes. The mean length followed by the standard deviation (SD) of 50 males and 32 females that emerged in the laboratory from field-collected cocoons, was 1.77 (0.119) and 1.80 (1.05) mm respectively, a sex ratio of 1 male to 0.64 females. A previous collection of 105 adults emerged in the laboratory in 1986 gave 52 males and 53 females or a 1:1 ratio. In 1987, sex ratios of emerging females in the field at the Bodnaryk site for 98 adults was 1 male to 1.8 females. There appears to be considerable variation in sex ratio of emerged adults suggesting that actual sex ratio should be based on the pupae before emergence.

1.2 The egg

The unlaid egg (oocyte) is elongate with a stalk (Fig. 4A.). The stalk is less noticeable once the egg is laid. Size of 25 eggs (mm) dissected from each of five females were as follows:

Fig. 3. A female P. penetrans resting on a wheat head (actual size about 2 mm).

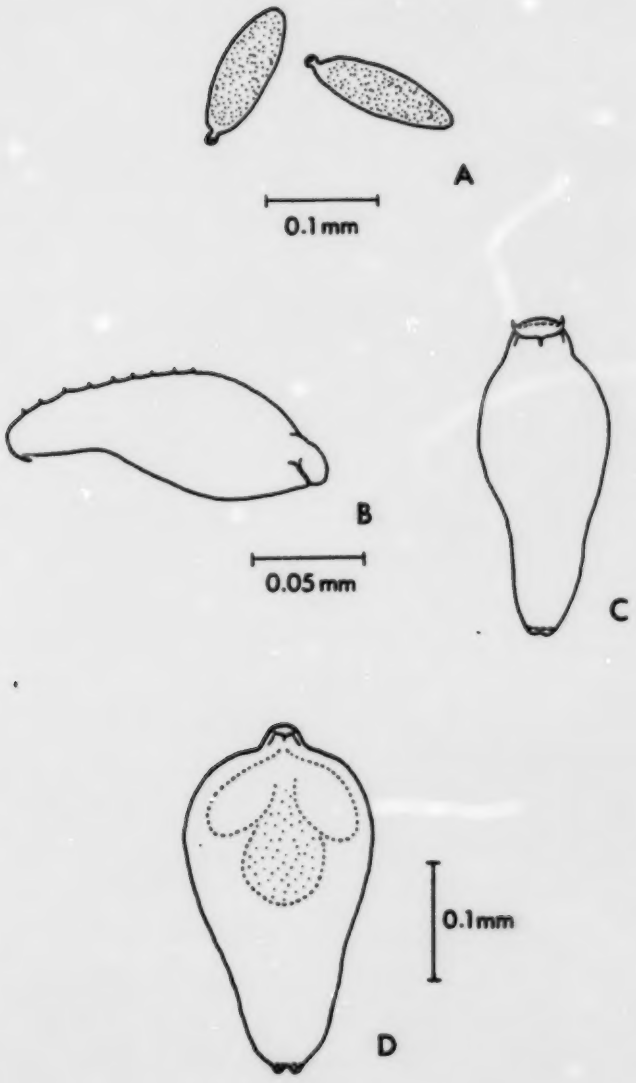

Fig. 4. Egg and larval stages of P. penetrans: A, eggs dissected from female; B, lateral and C, ventral views of early first instar larva; D, ventral view of a late first instar P. penetrans larva; stippled area indicates gut filled with a dense pale yellow substance;

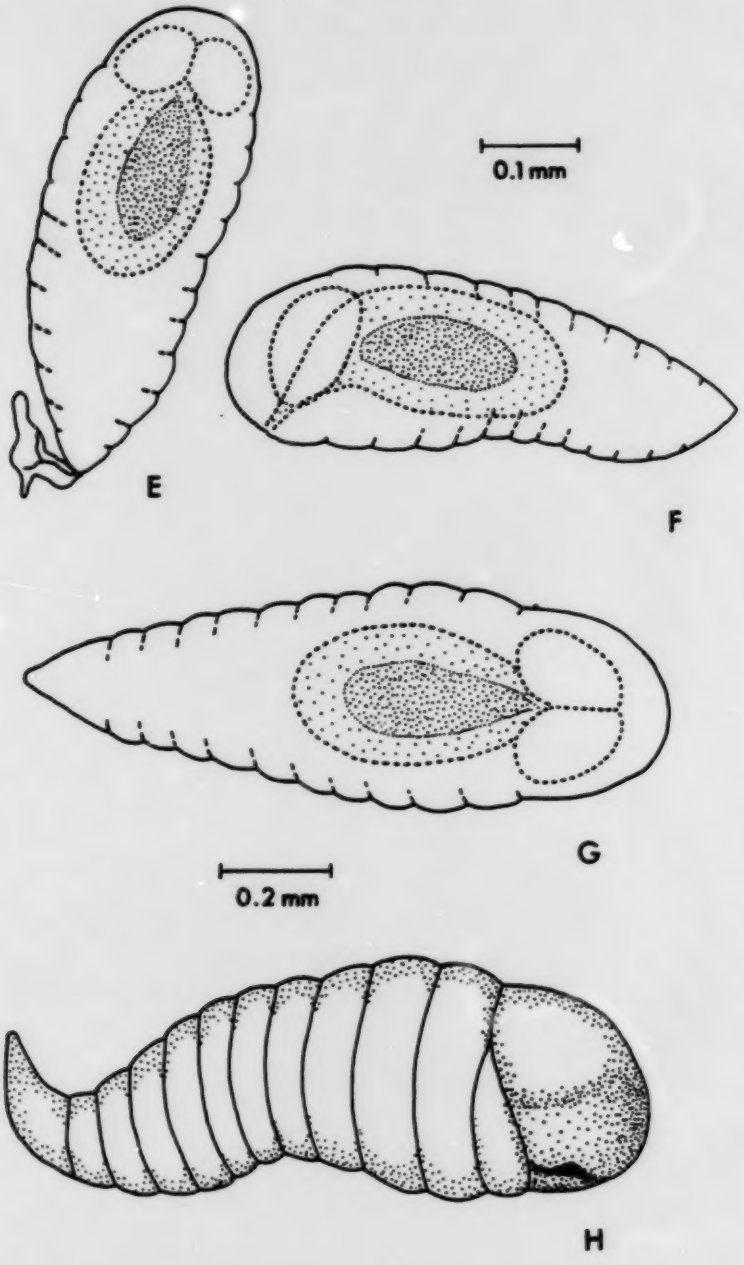

Fig. 4. (con't) E, dorsal and F, lateral view of an early second instar larva. Dorsal view shows membrane-like structure attached to the end of abdomen. Dense orange core of gut indicated by stippled area. In lateral view, note esophagus and its termination in the mouth region. G, dorsal view of late second instar larva showing organs (brain lobes and gut) seen through body wall; H, 3 dimensional lateral view of late second instar larva with characteristic tail curl.

Female No.	Length (SD)	Width (SD)
1	0.130 (0.005)	0.035 (0.004)
2	0.132 (0.010)	0.037 (0.004)
3	0.127 (0.006)	0.032 (0.003)
4	0.131 (0.006)	0.042 (0.003)
5	0.136 (0.008)	0.042 (0.004)
Mean	0.131 (0.008)	0.038 (0.004)

P. penetrans eggs that were laid in, and dissected from midge eggs (Fig. 5) were considerably shorter than unlaid eggs and about the same width. The length and width of 25 laid eggs were 0.105 (0.008) and 0.041 (0.006) mm respectively.

1.3 The larvae

Since measurements (HCW, HCL and BL) were not initiated until larvae were collected from the soil in September in 1986, these data were not used because the 1987 work showed that most first instar larvae had attained their full growth by September. In 1987, measurements began soon after parasite larvae began hatching in wheat midge in the wheat heads and a much larger sample was obtained than in the previous year. The 1987 work showed that unlike the second instar, the HCW of the first instar larva did not change in relation to body length. Since the ratio of the two parameters was different in the different instars cluster analysis was inappropriate as a method of analysis. Instead a frequency distribution was obtained for the 693 HCW measurements. This showed that there was a clear separation of first and second instars (Figs. 6 and 7). There was no indication of a third instar based on HCW. However, early and late stages of the first and second instar larvae were distinguishable (Figs. 4E-F and G-H) in shape and appearance so that this designation was made for convenience in describing the transition from one growth stage to the next throughout the year.

1.3.1 The first instar

First instar larvae began to hatch about the second week in July in the field. These newly hatched larvae have a prominent head but are transparent and

Fig. 5. P. penetrans eggs (e) laid in host wheat midge eggs. (Actual size of parasite eggs about 0.10 mm).

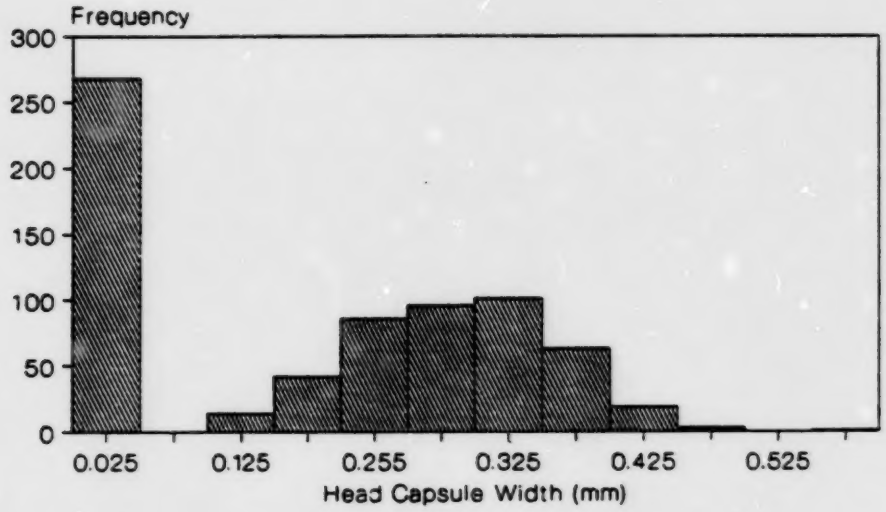

Fig. 6. Frequency distribution of head capsule widths of 693 P. penetrans larvae.

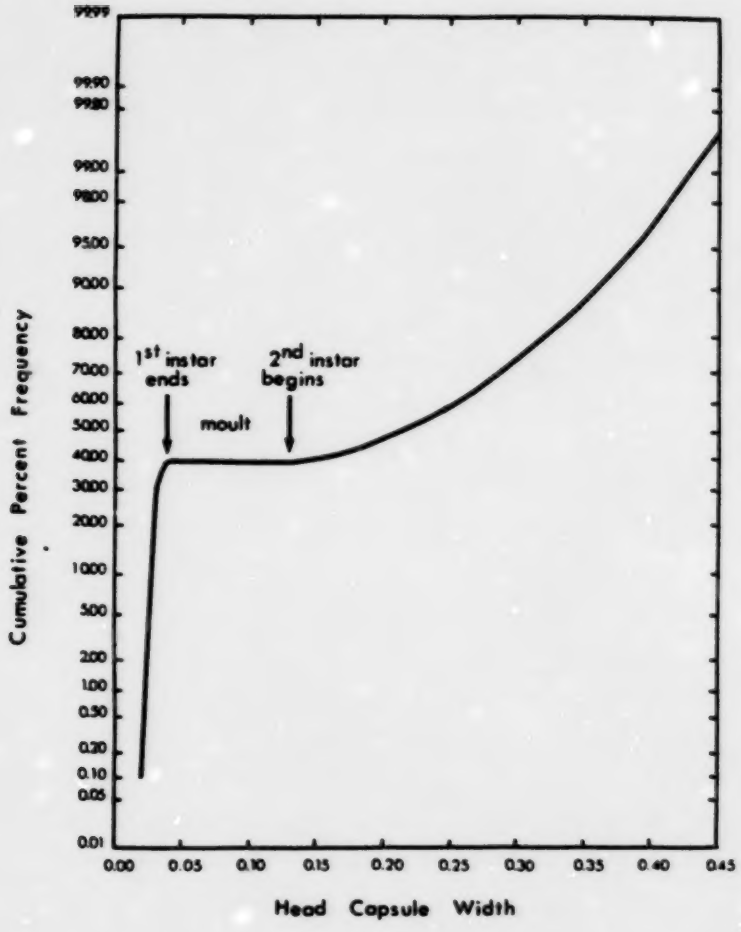

Fig. 7. Cumulative frequency distribution of head capsule width showing the end of the first instar and beginning of the second instar.

difficult to see (Fig. 4B and C). The body widens slightly behind the head and tapers to the end of the abdomen. About 11 segments may be visible in the newly hatched larva; a small spine or bristle mark each segment dorsally (Fig. 4B). A pair of small terminal ventrally curving hooks are seen on the abdomen (Fig. 4B). The tentorium is evident within the head in ventral view. Small sickle-shaped widely spaced mandibles that move independently are also evident (Fig. 4C). These may be used to breakout of the egg or to eliminate competitors since cases of superparasitism have been observed for only a short period after parasite eggs hatch. Subsequently only one parasite per host is found.

The first instar is tear-drop shaped (Fig. 4D). Although the body length increases from about 0.144 to 0.302 mm the width of the head capsule remains constant (Fig. 8). At this stage, segmentation and bristles on the dorsum are no longer evident. The caudal hooks are still present. The gut, filled with a pale yellowish substance can be seen through the body wall. The larger more opaque larva is therefore more easily seen when dissected from the host. A pair of small internal lobes, probably the brain, can be seen arising from behind the head and abutting the gut (Fig. 4D). The sickle-shaped mandibles are still present.

1.3.2 The second instar larva

The shape of the early second instar larva is distinctly different from the first instar. The head no longer protrudes anteriorally and no caudal hooks are present (Figs. 4E and F). There is also a marked increase in HCW (Figs. 6 and 7). The HCW, HCL and BL of five larvae from cocoons on September 2 were 0.146 (0.018), 0.073 (0.025) and 0.494 (0.53) mm respectively. The body is long relative to the head width; usually 11 body segments are evident with the thoracic segments being most prominent. Frequently, a transparent membrane-like structure is attached to the end of the abdomen and is evident just after the larva is dissected from the host (Fig. 4E). When the larva is placed in solution the membrane detaches from the larva.

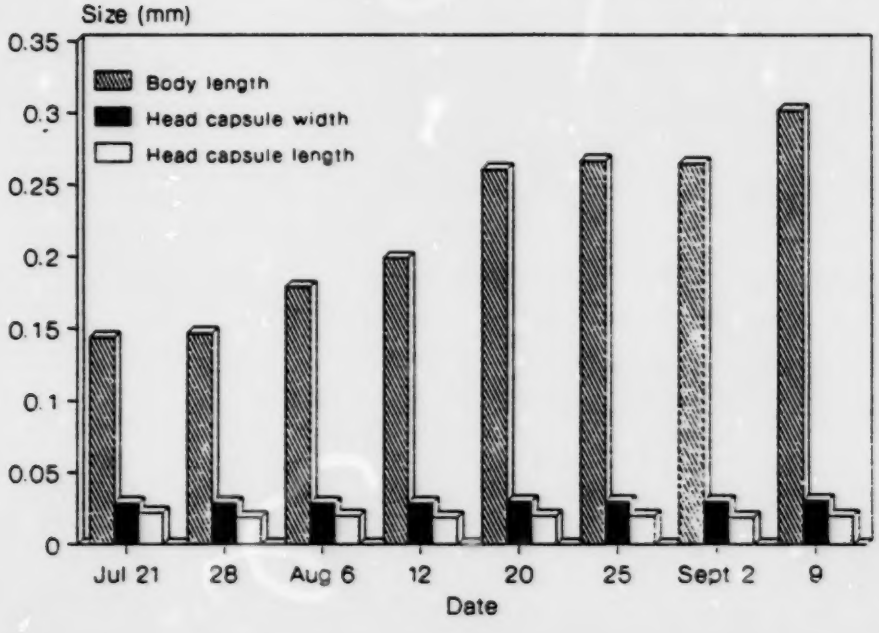

Fig. 8. Changes in body length of first instar larvae developing in midge in wheat heads. Head capsule width and length remain constant.

Through the body vall, gut and brain lobes can be seen. The gut has a central core filled with dense orange material. The esophagus can often be seen joining the gut and mouth region ventrally (Fig. 4F). No mouth parts vere observed in live larvae or cleared vhole mounts but cross-sections of fixed and stained larvae revealed a small pair of mandibles.

The late second instar larva is longer, vider and more robust than the early second instar. On May 4, 1987 the early second instar had attained a HCW, HCL and BL of 0.188 (0.025;n=29), 0.074 (0.016;n=27) and 0.514 (0.075;n=36) mm respectively. By June 2, they had reached their full growth with a mean HCW, HCL, and BL of 0.338 (0.053;n=22), 0.261 (0.042;n=21) and 1.052 (0.25;n=25) mm (Figs. 4, 5 and 6).

Segmentation is more evident with 11 body segments counted dorsally and 12 ventrally (Fig 4H). Larvae newly dissected from the host typically have the last two abdominal segments curled (Fig. 4H). Through the body vall orange gut and brain lobes can be seen (Fig. 4G). Percursal antennal and mouth part disks are evident as more or less circular areas on the head (Fig. 9). In some specimens there were as many as six of these disks evident.

1.4 The prepupa and pupa

The third instar transforms to the prepupa (Fig. 10A and B). The prepupa can first be seen within the integument of the late second instar larva. The body measurements (not included with larval measurements) of a limited number of prepupa (n=10) were 0.330 (0.086) and 1.403 (0.300) mm for HCW and BL respectively. The antennal disks are evident as definite protuberances. The mouth area is defined and bordered by two sclerotized hook-like process which appear to be the precursal mandibles. They are referred to as mandibles by Kutter (1934) in his description of the prepupa of Pirene graminae Hal., a parasite of Contarinia pisi Winn.

A large yellovish food pellet, taking up most of the gut area, is seen through the body vall. The terminal segment of the abdomen bears four spines

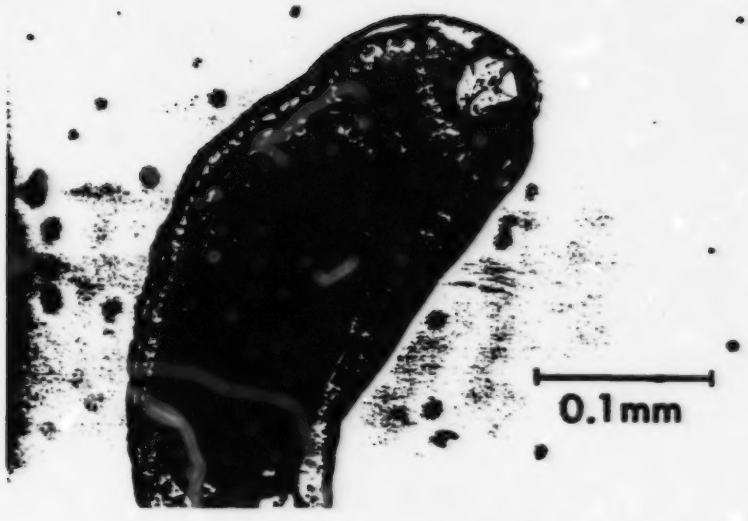

Fig. 9. Mid-late second instar P. penetrans larva showing light disk area of precursal antennae.

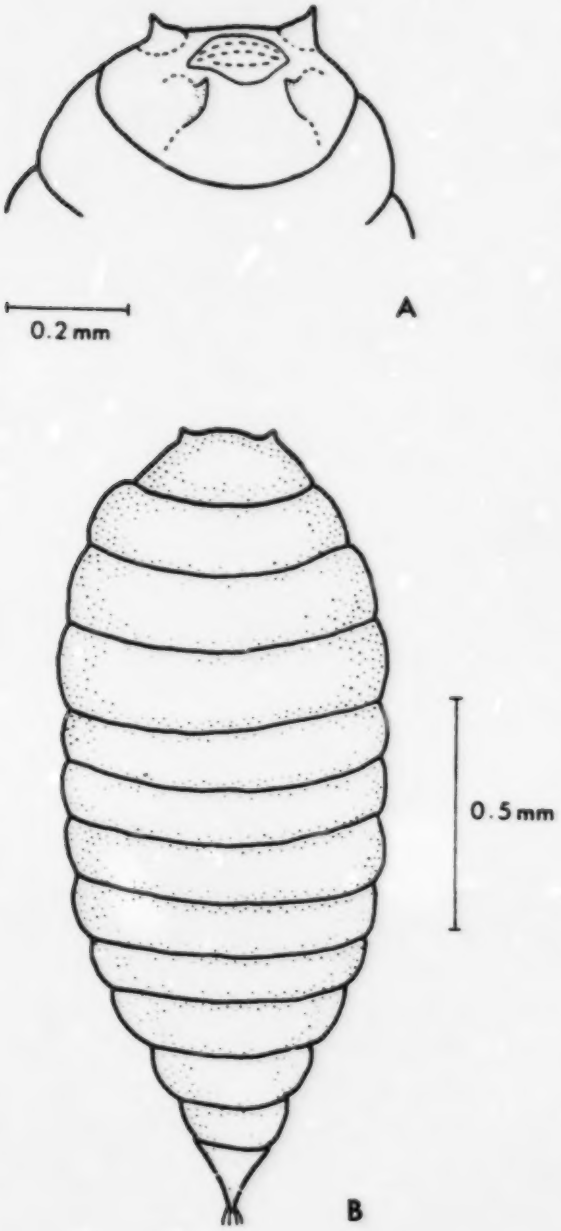

Fig. 10. A, Prepupa of P. penetrans showing head region; B, diagramatic dorsal view; note terminal spines.

similar to those described by Kutter for P. graminae.

The pupa of P. penetrans is about the same size as the adult (Fig. 11). In its early stages of development it is white and still retains a yellowish pellet of food in the gut carried over from the prepupa. The pupa gradually darkens as it approaches maturity. The pupal stage lasts about two weeks in the field but too few individuals in the pupal stage were found in soil samples to determine the duration of the pupal period. Pupation takes place in an elongate cocoon that is spun by the parasitized host larva before it is immobilized by the parasite larva. The prepupal and pupal stages develop within the cocoon.

2.0 Seasonal development and percent parasitism

2.1 Spring survey - 1987

The 17 fields that were surveyed in the Veregin district had an average cocoon population of 6092 (range 988 to 36364)/m^2 well above the economic threshold of about 700/m^2 (Table 1). Parasitism ranged from 0 to 44 percent with estimated densities of parasitized larvae averaging 398 (range 0-1581)/m^2.

Most parasite larvae were in the early second instar. The mean HCW, HCL and BL also placed the larval parasites in the early second instar (Table 1). The percent composition of different stages of 43 parasite larvae were 14, 70, and 16 for first instar, early second instar and late second instar respectively. Therefore, most parasite larvae overvintered in the early second instar.

2.2 Seasonal development of P. penetrans and percent parasitism of soil-collected midge larvae

The mean density of cocooned midge larvae on May 4 was estimated at 12945/m^2 (6.55/sample unit). This population remained about the same until June 16 and then a significant ($P<0.05$) decrease in the midge population occurred between June 16 and June 25 (Fig. 12). This decrease may have been due to mortality

Fig. 11. Pupa of P. penetrans. Note yellowish food pellet (actual size about 2 mm).

Table 1. Density of wheat midge larvae and its parasite Pirene penetrans in soil, in fields sampled in RM's 243 and 273, May 4, 1987.

Sample no.	RM no.	Section township and range	Midge mean larvae per soil core	Midge density per m2	Mean parasitized larvae per soil core	Parasite density per m2	% Parasitism	Mean size parasitoid larvae (mm) HCW	HCL	BL	No. measured
1	243	SW28-27-1	2.9	5731	0	0	0	--	--	--	
2	243	SE29-27-1	3.3	6522	0.1	100	3.0	0.171	0.067	0.489	1
3	273	SE5-28-1	2.2	4348	0	0	0	--	--	--	
4	273	SE8-28-1	0.8	1581	0	0	0	--	--	--	
5	273	NE16-28-1	1.5	1976	0	0	0	--	--	--	
6	273	SE21-28-1	4.4	8696	0	0	0	--	--	--	
7	273	NE28-28-1	18.4	36364	5.9	1166.0	32	0.191	0.080	0.563	14
8	273	SW34-28-1	3.8	7585	0	0	0	--	--	--	
9	273	SE33-28-1	0.5	988	0.1	198	20	0.214	0.090	0.489	1
10	273	SE33-28-1	0.9	1779	0.1	198	11	0.190	0.095	0.523	1
11	273	NE16-28-1	5.5	10810	0.6	1186	12	0.029	0.014	0.295	1
12	273	SW15-28-1	0.7	1383	0.2	395	29	0.147	0.062	0.504	2
13	273	SW3-28-1	2.2	4348	0.8	1581	36	0.164	0.073	0.564	8
14	273	NW11-28-1	1.2	2372	0.08	165	8	0.195	0.076	0.451	1
15	273	NW34-28-1	0.9	1778	0.4	790	44	0.150	0.070	0.517	4
16	273	NW22-28-1	2.2	4348	0	0	0	--	--	--	
17	273	NW17-28-32	1.5	2964	0.5	988	33	0.103	0.08	0.427	3
Mean			3.1	6092	0.5	398	13.4	0.155	0.071	0.482	

[1] Estimated instar composition of 43 parasite larvae was 13.9, 69.8 and 16.3 for the 1st, early and late second instar larvae, respectively.

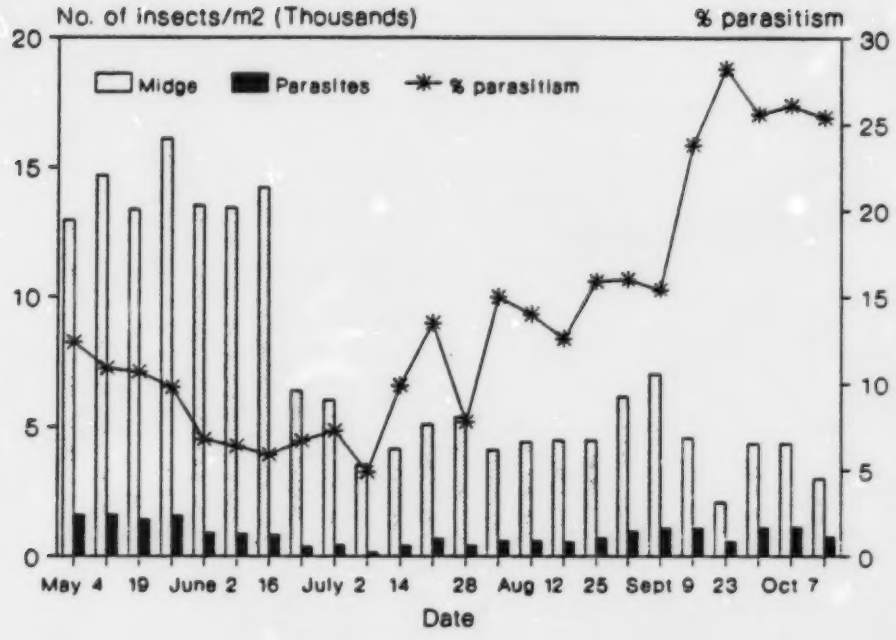

Fig. 12. Populations of wheat midge, parasitized midge larvae and percent parasitism from May to October in the soil at the Bodnaryk site, 1987.

associated with pupation since few midge pupae were found in samples on or after June 25.

The initial population of parasitized midge larvae decreased significantly ($P<0.005$) from 1606/m^2 (0.81/sample unit) on May 4 to 840/m^2 (0.42/sample unit) on June 16. No accompanying decrease of host density between May 4 and June 16 was observed. This was apparently due to the death of parasite larvae within the host midge larvae which remained alive. Dissections revealed the presence of dead parasites or their remnants. The breakdown of parasite larvae within the host often was almost complete when the host was dissected. This factor resulted in a decline in the percent parasitism from 12% on May 4, to 6% on June 16 (Fig. 12). No indication of encapsulation of the parasite larvae by the host was evident.

After September 9, there was a sharp increase in percent parasitism which remained consistently high from September 9 until October 14. This increase was attributed to an increase of parasitized larvae entering the soil after September 9, when the crop was harvested. Higher mortality of unparasitized larvae may also have been a factor since they left the heads before the parasitized larvae (see Fig. 16). During the season, parasitism increased from 12% on May 4 to about 25% on October 14 (Fig. 12).

As mentioned previously, most larvae overwintered in the early second instar and then grew rapidly until reaching full size about June 2 (Fig. 13). Thereafter some emergence of hosts and parasites occurred during July but a portion of the population, both parasitized and unparasitized larvae, remained in or re-entered extended diapause (Fig. 13) to be carried over to the next season.

Composition of growth stages from spring to autumn at the Bodnaryk site is shown in Fig. 14. In early May the parasite population was composed mostly of early second instar larvae with a few first instar larvae still present (Fig. 14). These grow rapidly during May giving rise to late second instar larvae which constitute about 100% of the population by early June (Fig. 14). Those larvae (second instar stage), remaining in extended diapause, are found

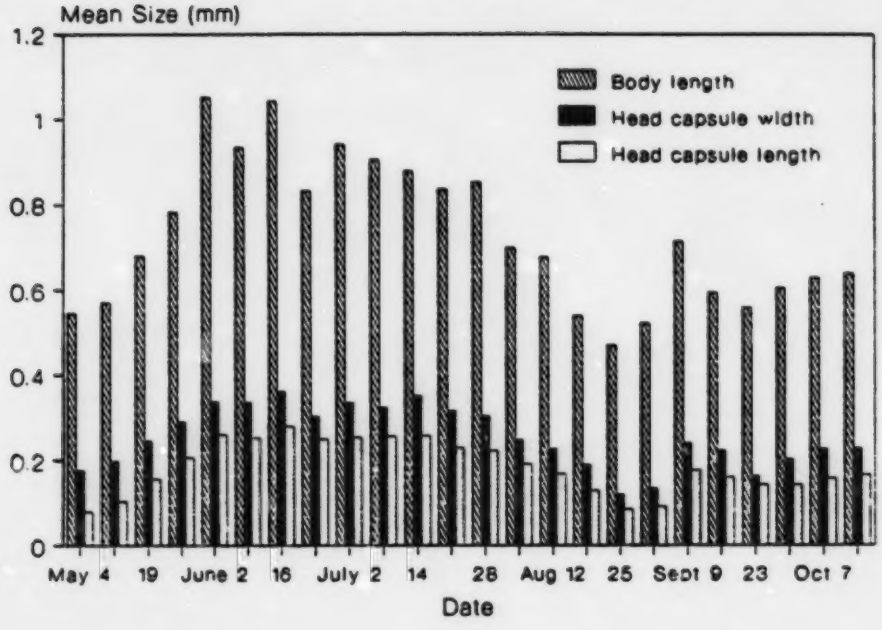

Fig. 13. Changes in body length, head capsule width and head capsule length of P. penetrans larvae dissected from soil-collected midge larvae at the Bodnaryk site, 1987.

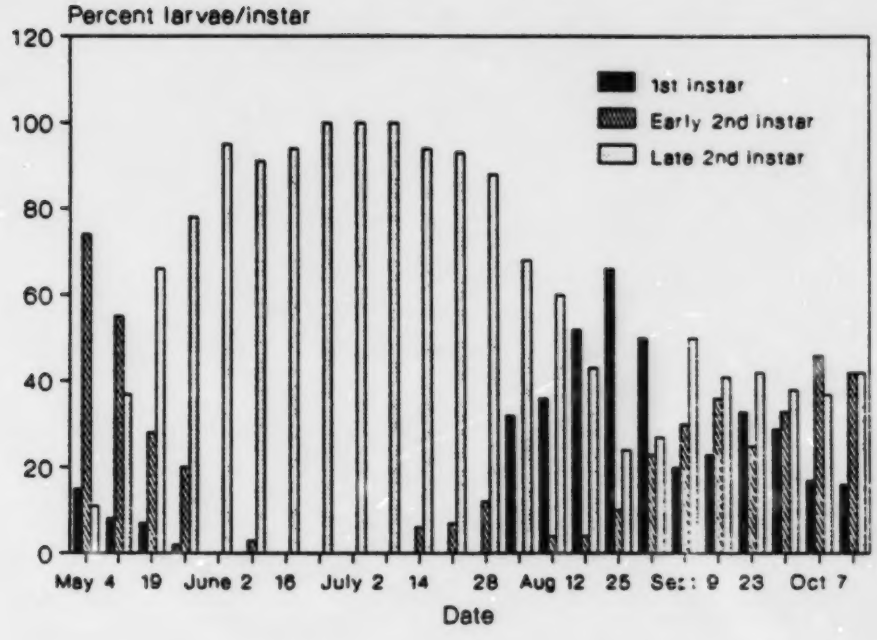

Fig. 14. Composition of P. penetrans larvae in different growth stages in percent from May to October 1987 at the Bodnaryk site, 1987.

throughout July along with a few early seconds which also appeared to have remained in diapause. First instar larvae began dropping from the heads about August 6 and were then found in soil samples. The rate of growth of parasite larvae from the heads and soil was followed separately (Figs. 8 and 13). In the soil an increase in numbers of early second instar larvae was seen about September 2 indicating a moult from the first to second instar.

In the heads, very few second instars had been found by September 9, when the crop was harvested. Following this, the larvae left the heads to enter the soil and an increase in early second instars occurred until October 14 when the last soil sample was made and the mean daily soil temperature at 5-10 cm had decreased to 4.5°C. This indicated that the majority of parasites produced in 1987 would overwinter in the second instar along with some late second instar larvae that had been carried over from the 1986 population in extended diapause.

Midge eggs were found in the heads from July 2 until August 6 and midge larvae from July 7 to September 9 (Fig. 15). Midge eggs and larvae were dissected each week (see Experimental Methods section 3.1). The highest number of parasitized eggs were found from July 2 and 7 (Fig. 15), thereafter parasite eggs began to hatch. The percent parasitism of larvae increased steadily after larvae began leaving the head. This was because parasitized larvae left the heads first so that a higher percentage of the larvae that remained were parasitized (Fig. 16).

2.3 Depth of parasitized and unparasitized larvae

Soil samples showed that significantly more larvae, both parasitized and unparasitized were found at the two deepest sampling depths (Table 2). Also significantly more parasitized larvae were found at 5.2-7.6 cms than lower or higher. No significant difference was found in the proportion of midge-parasitized larvae at different depths although there appeared to be a trend for higher parasitism at the deepest sampling depth.

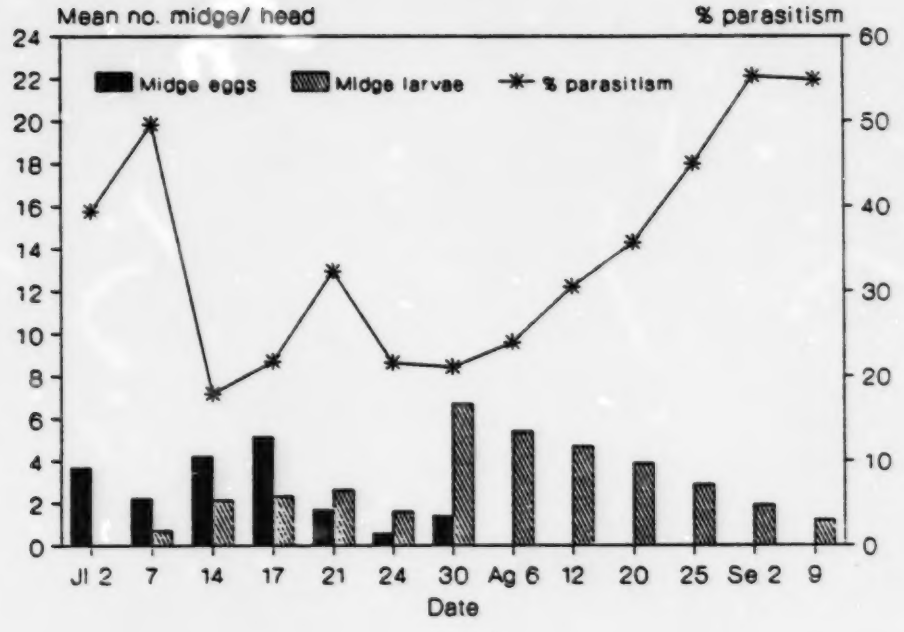

Fig. 15. Mean numbers of midge eggs and larvae dissected from wheat heads and percent parasitism by P. penetrans, July 2 - September 9 at the Bodnaryk site, 1987.

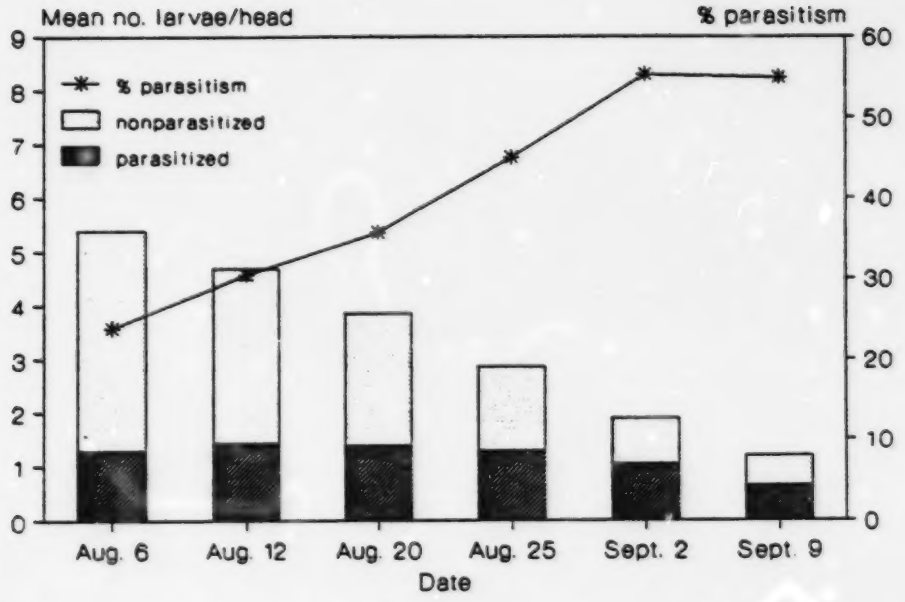

Fig. 16. Decrease in numbers of non-parasitized and parasitized wheat midge larvae and percent parasitism after August 6, at the Bodnaryk site, 1987.

Table 2. Mean numbers of parasitized and unparasitized midge larvae as determined by soil samples at four different depths.

Depth (cm)	Parasitized larvae	Unparasitized larvae	Total	Percent parasitized
0 - 2.5	0.72 a	9.97 a	10.69 a	5.95 a
2.6 - 5.1	1.28 a	15.81 a	17.09 a	4.65 a
5.2 - 7.6	1.90 a	24.06 b	25.97 b	6.11 a
7.7 - 10.2	2.31 b	21.06 a	23.38 a	9.89 a

Means followed by the same letter are not significantly different, $P<0.05$

Too few midge or parasite pupae were found to compare the depth of pupation of parasite and host.

2.4 Emergence of midge and parasites in the field

Emergence of small numbers of both midge and P. penetrans occurred between June 25 and July 2 (Fig. 17). The peak emergence for midge occurred July 14-21. Emergence of parasites was greater from July 2-8 and July 21-28 than in mid-season. The numbers of male and female midge emerging was 187 males and 280 females, or based on a trap area of 135.23 cm^2 and 32 traps, an emergence of 432 and 647/m^2 for males and females respectively. Between July 2 and August 6 the midge population in the soil dropped from 6052 to 4125/m^2. Therefore, approximately 56% of this decrease could be attributed to emergence, the remainder to unexplained mortality.

There were 33 male and 68 female parasite adults or a seasonal emergence of 76 males or 157 female/m^2. This emergence could not be detected as a drop in numbers of parasitized larvae since the parasite population was about 400/m^2 on July 2 and about the same on July 28. About 5400 midge larvae/m^2, both parasitized and unparasitized, remained in extended diapause and did not emerge (Fig. 12).

2.5 Parasitism in the field in relation to location of midge eggs in florets

Analysis of variance showed that spikelet position within a head was not related to midge egg distribution, midge egg mortality or parasitism. Within the floret significantly more eggs were usually found on the palea than on other floret structures but this was quite variable (Table 3). The majority of living eggs were also found on the palea but there appeared to be no trend in distribution of dead eggs within the florets. In general parasitism was highest where most midge eggs were found. There was no trend in location of dead parasitized eggs.

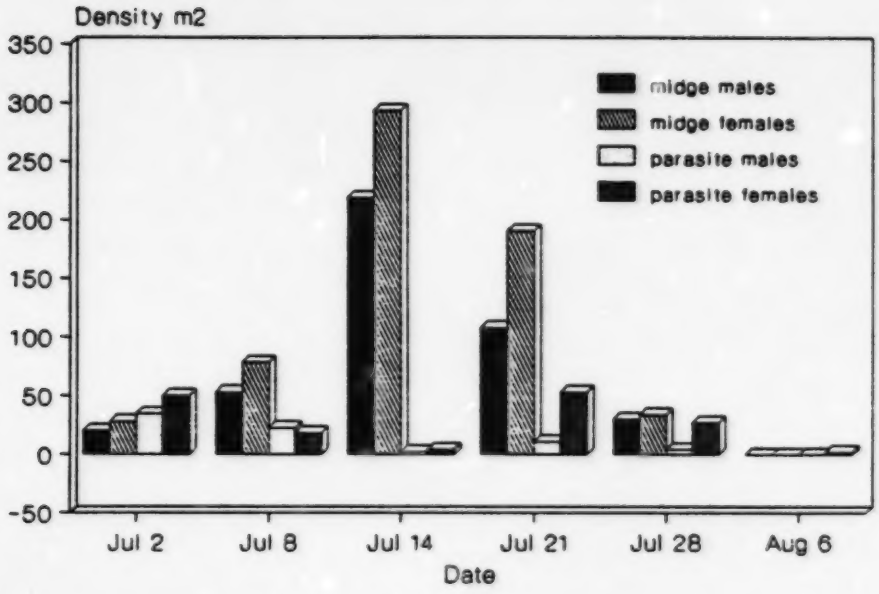

Fig. 17. Density of midge and P. penetrans adults emerging from soil at the Bodnaryk site, 1987.

Table 3. Analysis of variance for numbers of midge eggs parasitized and not parasitized, alive and dead on various parts of the floret for counts made at weekly intervals

Date	Total eggs	Eggs alive	Eggs dead	Eggs not parasitized	Eggs parasitized	Eggs alive not parasitized	Eggs alive parasitized	Eggs dead not parasitized	Eggs dead parasitized
July 7	Sig more on pa	Sig more on pa	No sig diff	Sig more on pa	Sig more on pa	Sig more on pa	Sig more on pa & gi	No sig diff	No sig diff
July 14	Sig more on pa, gi & lo	Sig more on pa, lo ra, & gi	Sig more on gi	Sig more on gi & pa	No sig diff	Sig more on pa, ra, lo	No sig diff	Sig more on gi	No sig diff
July 17	Sig more on pa	Sig more on pa	No sig diff	Sig more on pa	Sig more pa,gi,lo ra, & go	Sig more on pa	No sig diff	No sig diff	No sig diff
July 21	More on lo	No sig diff	No sig diff	No sig diff	Sig more on lo	No sig diff	No sig diff	No sig diff	No sig diff
July 28	Sig more on pa	Sig more on pa	Sig more on pa	Sig more on pa,lo gi & go	More on pa,lo & gi	Sig more on pa	Sig more on pa	No sig diff	Sig more on pa,lo gi,li & go

go=outside glume; gi=inside glume; lo=outside lemma; li=inside lemma;pa=palea; ra=rachis.

A total of 1586 larvae were taken from wheat heads and dissected between July 7 and September 9. (Table 4). Heads of both primary and secondary tillers examined, showed that mortality of both unparasitized and parasitized larvae was low but as determined by chi square analysis, lower in parasitized than unparasitized larvae for all tillers (χ^2 = 22; P<0.01) and for primary tillers (χ^2 = 6.57; P=<0.02). In both primary and secondary tillers the overall percent parasitism for all dates was the same, 31%.

3.0 Seasonal and diurnal activity of P. penetrans and midge adults in the field

3.1 Seasonal captures on "sticky board" traps

There was no significant effect of direction P>0.05 for either the mean number of midge or parasites caught on sticky traps and with top and bottom catches combined (Tables 5 and 6) (after analysis of both original data and after transformation by $\sqrt{x + 1}$). Peak catches were made from July 14-21 for both midge and parasites; a second smaller peak occurred toward the end of the season. Significantly more (P<0.05) midge, mostly females, were caught in lower boards (Table 5). Conversely, significantly more parasites were caught on the upper boards. Again this was true for both original and transformed data ($\sqrt{x + 1}$). The midge adults are therefore more active in the lower canopy near the ground while the parasites are more active in the upper canopy. Both midge females and parasite females were caught in relatively high numbers late in the flight season July 28 - August 6 (Tables 5 and 6). Over the same period, few males of the host or parasite were captured. The reason for this difference in sexes is not known.

3.2 Diurnal activity

Experiment 1 - For midge trapped, significant differences (P<0.05) were found between the cloudy day, July 22, and the sunny day July 23 (χ^2=5.45), and in direction trap face (χ^2=4.48); highly significant differences were found between time of day (χ^2=40.34) and sex (χ^2=23.52). Fewer midge were trapped on the cloudy day (July 22) as compared to the sunny day (July 23) and more during the

Table 4. Midge larvae within wheat head, parasitism and mortality, Bodnaryk's 1987

Larvae from	Total	No. dead	No. parasitized	No. dead and parasitized
All tillers	1586	96 (6)	489 (31)	9 (0.6)
Primary tillers	604	30 (5)	188 (31)	3 (0.5)
Secondary tillers	982	66 (7)	301 (31)	6 (0.6)

Numbers in brackets are % of total larvae

Table 5. Seasonal captures of wheat midge adults on "sticky board" traps at the Glute site - 1987

Trap Direction	Trap Position	July 8		July 14		July 21		July 28		Aug. 6		Mean	
		males	females	males	females	males	females	males	females	males	females	males	females
North	High	0	0	0	3.00	0.25	15.50	0	8.50	0	7.75	0.05	6.95
	Low	0	2.50	0	15.25	0.75	90.25	0.75	8.75	0.50	13.50	0.40	26.05
South	High	0	0	0.25	1.50	0	26.25	0	3.75	0	8.25	0.05	7.95
	Low	0	0	0.50	10.75	0.50	82.25	0.25	5.75	0.25	12.50	0.30	22.25
East	High	0	0	0	3.75	0	10.25	0.25	5.00	0	5.75	0.05	4.95
	Low	0	0	1.25	13.75	0.75	65.00	0	6.25	0.50	18.25	0.50	20.65
West	High	0	0	0	3.25	0	13.25	0.25	6.00	0	8.25	0.05	6.15
	Low	0	0	0.75	22.75	1.00	88.25	0	6.50	0	13.75	0.35	26.25
All Direction Mean	High	0	0	0.06	2.88	0.06	16.31	0.13	5.81	0	7.50	0.05	6.50
	Low	0	0.06	0.63	15.63	0.75	81.44	0.25	6.81	0.31	14.50	0.39	23.69

Table 6. Seasonal captures of *Pirene penetrans* adults on "sticky board" traps at the Glute site - 1987

Trap Direction	Trap Position	July 8		July 14		July 21		July 28		Aug. 6		Mean	
		males	females	males	females	males	females	males	females	males	females	males	females
North	High	1.00	0.25	0	0.50	7.00	3.75	1.25	0.25	0.25	5.50	1.90	2.05
	Low	0.25	0.25	0	0	1.25	1.00	0.25	0	0	4.75	0.35	1.20
South	High	0.25	0.25	0.25	0.25	1.25	2.00	2.00	0	0	5.75	0.75	1.65
	Low	0	0.25	0.25	0.25	1.50	2.25	0	0	0	3.75	0.35	1.30
East	High	0.50	0.25	0	0	10.50	5.00	5.50	1.25	0	7.75	3.30	2.85
	Low	0	0	0	0	2.00	1.75	1.00	0.25	0	3.25	0.60	1.05
West	High	1.50	0	0	0.25	1.75	2.50	0.75	0.50	0	8.50	0.60	2.35
	Low	0	0	0	0	2.00	0.75	0.75	0	0	2.50	0.55	0.65
All Direction Mean	High	0.81	0.19	0.06	0.25	5.13	3.31	2.38	0.50	0.06	6.88	1.64	2.22
	Low	0.06	0.12	0.06	0.06	1.69	1.43	0.50	0.60	0	3.56	0.46	1.05

evening from approximately 18:00-22:00 h (Fig. 18A and B). Fewer midge were trapped on boards facing west (Fig. 18C). No reason for this was apparent and no directional differences were found in weekly board catches (Tables 5 and 6).

For P. penetrans adults, differences in numbers trapped on different days (χ^2=36.00), time of day (χ^2=35.36), upper and lower canopy (χ^2=12.25), and direction (χ^2=9.43) were all highly significant (P<0.01). More were caught on the sunny day and during mid-day from about 10:00 to 14:00 hrs (Fig. 18A and B). There was no significant difference in the number of male and female parasites trapped. Similar to the midge, fewer parasites were trapped on boards facing west. Interaction between time of day and day was highly significant (χ^2=35.8) in that parasites were trapped during mid-day and more on the upper board than the lower board during the sunny day. In other words sunny weather increased the activity in the upper canopy.

Experiment 2 - More parasites were observed on heads during the sunny day (164) than during the cloudy day (105), a highly significant difference (χ^2=12.94;P<0.01). Significantly more were observed in the upper canopy both days (175 vs 94; χ^2=24.39;P<0.01). Also, there were highly significant interactions in their location in the canopy and the day (χ^2=12.88) and in the location in the canopy and time of day (χ^2=39.00). During the cloudy day (July 22), females were mostly in the upper canopy (Fig. 19A). During the sunny day (July 23), more were in the lower canopy until 12:30 and then began moving up to the upper canopy later in the day (Fig. 19B).

Egg-laying was also affected by the weather. During the cloudy day only 10% of the females on heads were ovipositing compared to 38% during the sunny day (Figs. 19C and D). During the cloudy day, ovipositing females were seen only in the upper canopy and mostly at 16:30 hrs when air temperature had risen to 15°C and illumination to about 5000 lux (Fig. 6). Also by this time, a light breeze was blowing. During the sunny day oviposition was first observed in the lower canopy at 10:30 when the air temperature was about 22°C (Fig. 18). From 14:30 to 16:30 the percentages of ovipositing females were about equal in upper and lower

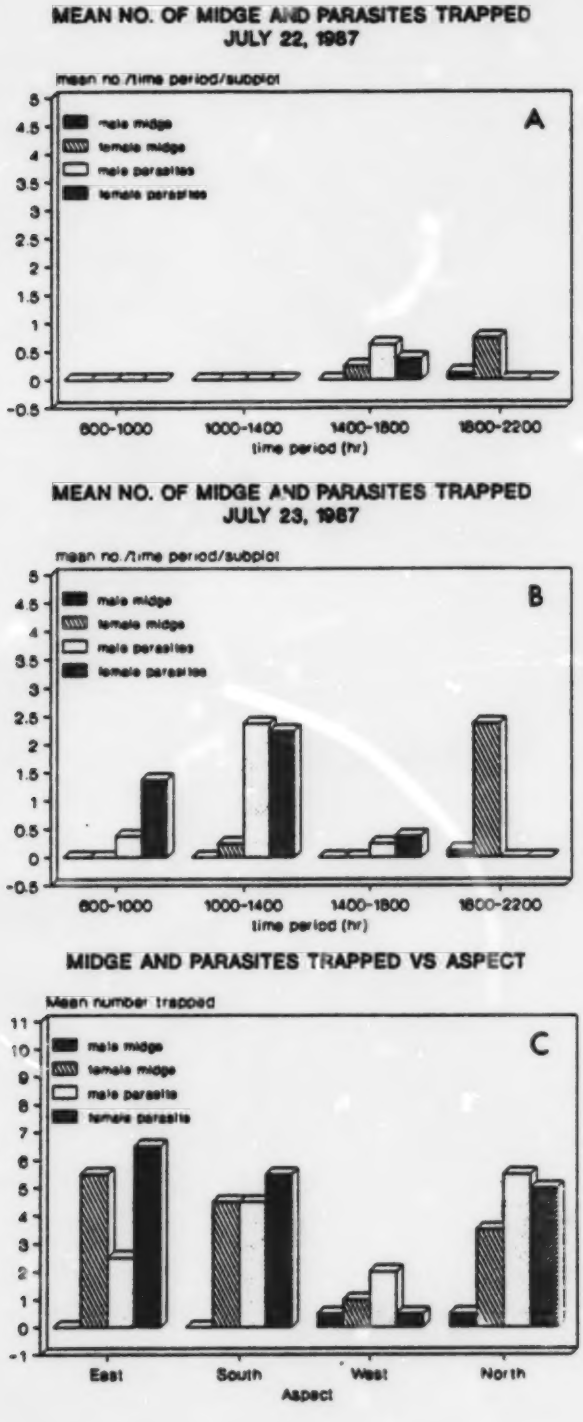

Fig. 18. Mean number of midge and P. penetrans adults trapped on "sticky board" traps during 4-hour periods: A, on July 22, a cloudy day; B, on July 23, a sunny day; C, on traps facing the four cardinal directions.

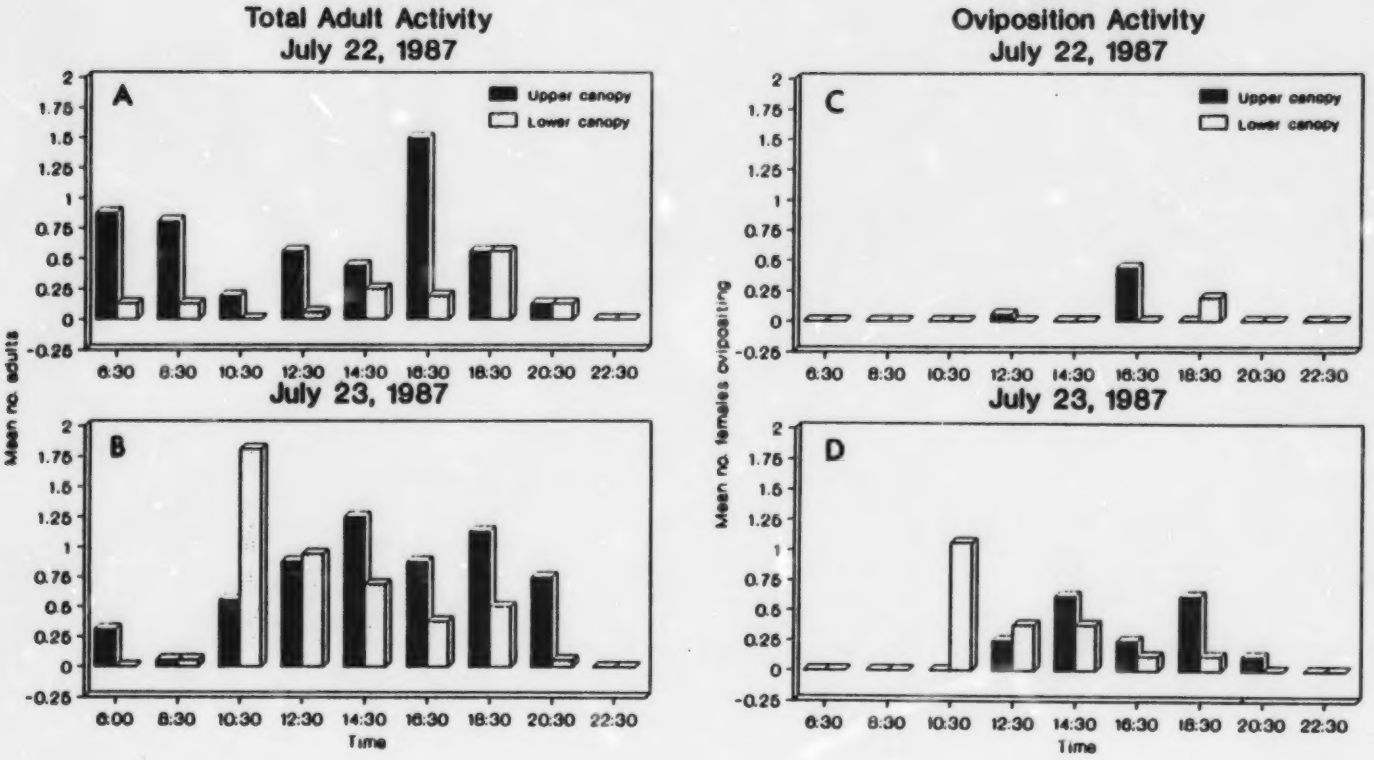

Fig. 19. Total activity of females of P. penetrans in the upper and lower canopy on a cloudy day (A), on a sunny day (B), and ovipositional activity on a cloudy (C), and a sunny (D), at the Glute site, 1987.

canopy and by 18:30 they were mostly in the upper canopy possibly in response to light.

Other environmental parameters that coincided with the observations on trapping and activity on wheat heads are given in Figs. 20 and 21 for July 22 and 23 respectively.

In general, activity was greater for parasites during the sunny day, mostly between 10:00 and 18:00 hours. Although some activity occurred even during cloudy rainy weather, the incidence of ovipositing females was much less. As expected midge females were mainly active during the evening after 18:00 hrs and particularly during the sunny day. At this time there was no breeze, temperature was about 16°C, and relative humidity at 75%.

Neither males on heads, nor matings were observed anywhere in the canopy during the study.

4.0 Behavior and development studies in the laboratory

4.1 Rearing of wheat midge

A continuous emergence of wheat midge adults was obtained by this method. Egg-laying was enhanced if adults were allowed to mature 2-3 days before being placed with a susceptible wheat head. Notes on numbers of midge adults and numbers of eggs laid are given in Table 7. Actual numbers of males and females were not determined but emergence of midge from 763 field-collected cocoons at 23°C in an earlier experiment (Doane unpublished) gave a ratio of .61 males to .39 females. If this ratio is used for the data of Table 7, then about 26 of the mean number of 67 midge were female and each laid about 1.7 eggs in a 12 h period.

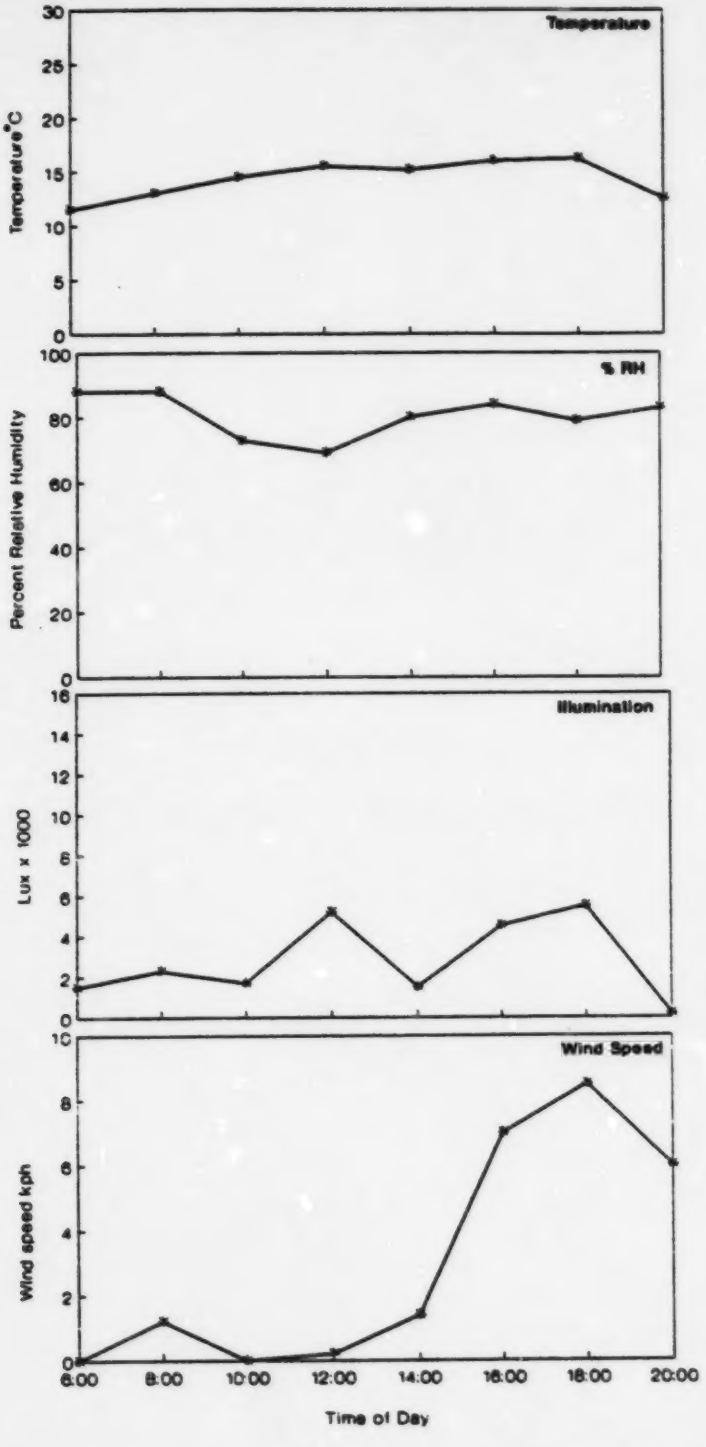

Fig. 20. Environmental parameters of temperature (°C), relative humidity (%), illumination (lux) and wind speed (kph) recorded every 2 h at the Glute site, July 22, 1987.

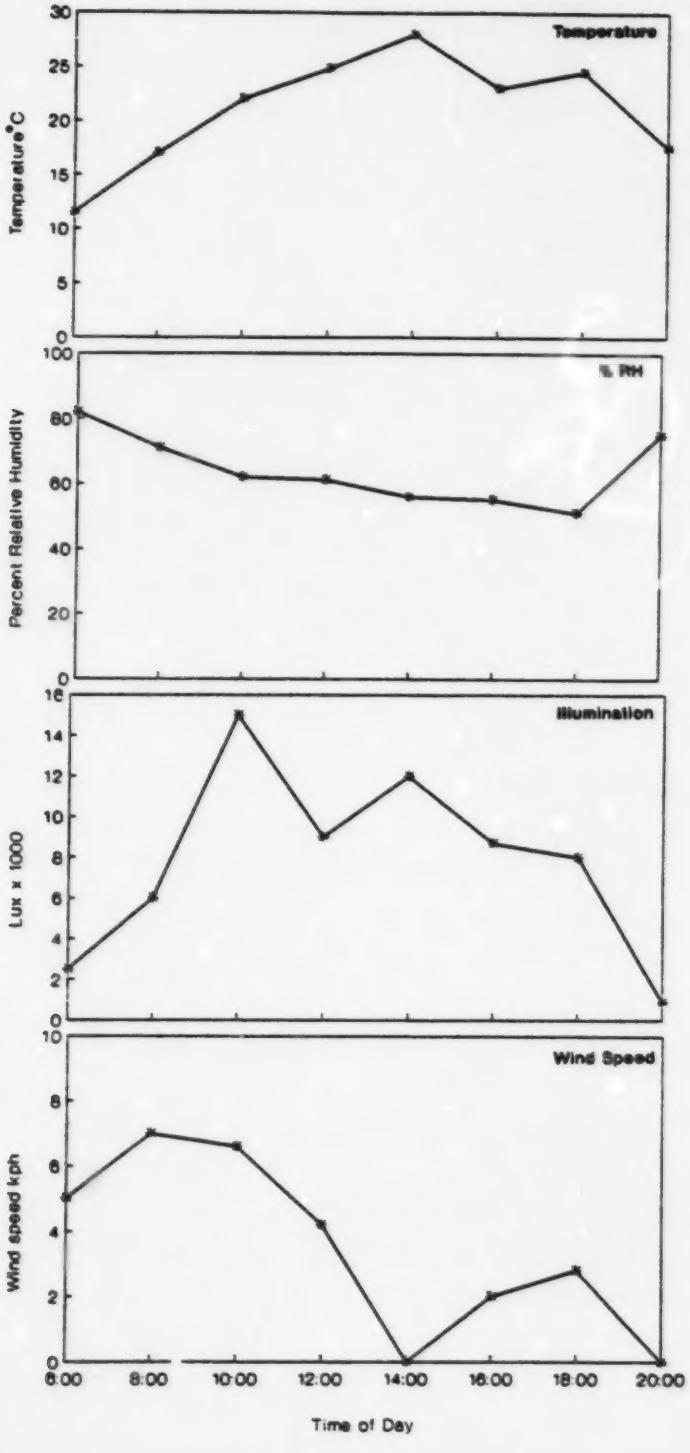

Fig. 21. Environmental parameters of temperature (°C), relative humidity (%), illumination (lux) and wind speed (kph) recorded every 2 h at the Glute site, July 23, 1987.

Table 7. Wheat midge oviposition in rearing cages

Date	No. eggs laid per head	No. midge in cage
28/09/87	68	40
5/10/87	27	20
16/11/87	34	22
9/12/87	24	30
13/12/87	15 110 50	110
14/12/87	34 37 50	115
13/1/88	25 101	110
14/1/88	42 33	110
25/1/88	24	50
Mean (SD)	45 (28)	67 (43)

* about 26 of which were female

4.2 Rearing of parasites

Parasites that emerged in rearing cones and were then transferred to parasite rearing cages would readily lay eggs in midge eggs (Fig. 22). A period of 2-3 days to allow parasite females to mature their eggs increased the likelihood of their oviposition. No parasite oviposition was obtained when parasite females were placed in large rearing cages with wheat heads infested with midge eggs. In small cages, parasite oviposition was more likely to occur if the female was placed directly on the head with a paint brush. The longevity of female parasites was also better in smaller cages probably because of the food and higher humidity. Both males and females were observed to feed from wicks saturated with sugar/honey solution. Females lived from 1-16 days after emergence (mean 6.16 $\pm$ 4.1; n=19). Some of these females oviposited during their lifetime. Males emerging on the same date as females died before the females. The longest period of time a male was found to live was 8 days.

4.3 Parasite development

P. penetrans eggs hatch at about the same time the host midge eggs hatch (Table 8). After 5 days at 23°C the majority of both midge eggs (up to 12 h old) and parasite eggs (up to 2 h old) had hatched. The size of newly hatched larvae was the same as mentioned under section 1.3).

Parasite eggs were frequently found in newly hatched midge larvae removed from wheat heads in the field. Also, cases of superparasitism with 2-4 parasite eggs/midge egg were common in the field but only in a few cases were observed in the laboratory. Early first instar midge larvae were found containing 2-3 newly hatched parasite larvae. However, in older first instar midge larvae, only one parasite larvae survived.

4.4 Parasite oviposition behavior

Observations of females during egg-laying showed that the following behavior was displayed.

Fig. 22. Female P. penetrans laying eggs into exposed orange wheat midge eggs.

Table 8. Parasite Development at 23°C in the laboratory

Days after parasite oviposition	Stage of midge development	Stage of parasite development
2 days	Midge in egg stage	Parasite in egg stage
4 days	Majority of midge eggs close to hatching. Some 1st instar larvae present.	Parasite eggs very close to hatching - some hatched. Parasite eggs found in some midge larvae and some 1st instar parasite larvae found in hatching midge eggs.
5 days	Majority of midge eggs hatched.	Majority of parasite eggs hatched.
6 days	Majority of midge eggs hatched.	Majority of parasite eggs hatched. Mean sizes for 12 early 1st instar larvae Body length = 0.124 ± 0.026 Head capsule width = 0.029 ± 0.004 Head capsule length = 0.019 ± 0.002

NOTE: Midge eggs ca. 12 hours old when exposed to parasites

a) Grooming - The female rubs her antennae with her forelegs and abdomen with her hindlegs when near or on the wheat head. This usually occurs briefly before she begins searching the head for eggs or during the search.

b) Abdomen flexing - This occurs when the female is on the head, a few seconds before searching begins.

c) Searching - In the majority of cases, searching begins immediately after the female is placed on the head but the behavior under a) and b) may occur first. During a search the female continually taps the wheat head with her antennae or waves them in the air. She fully extends her ovipositor to probe under the glumes, around the lemma and palea and along the rachis. Probing occurs continuously while she searches for eggs within the floret until she retracts her ovipositor to move to a new location. When probing under the glumes, she keeps her ovipositor extended while walking around the edge of the glume sometimes stopping to reach down as far as possible (Fig. 23). In these cases, she lies parallel to the glume edge.

The female parasite indiscriminately moves from spikelet to spikelet, either going up or down the wheat head, usually searching the whole head. She usually faces up when probing so that if moving down the head she will turn around and face up to probe. Females would continue searching for 2-3 hours when left on the head for that length of time.

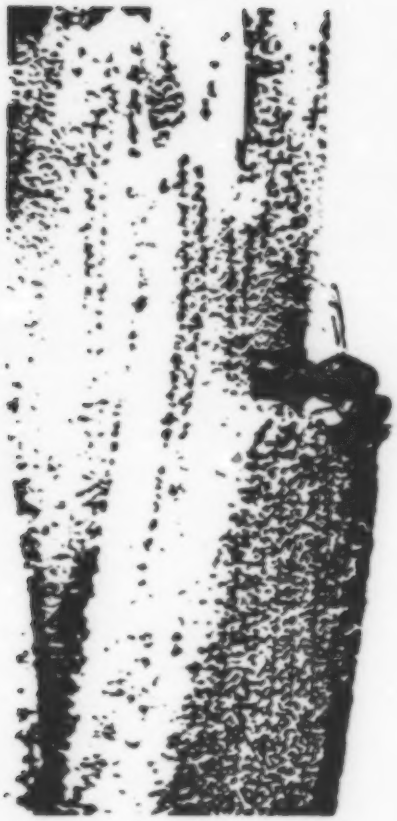

Fig. 23. Female P. penetrans probing behind a glume down into the floret.

d) Oviposition - When the female parasite locates midge eggs she immediately begins to oviposit, while remaining stationary with the ovipositor extended (Fig. 22). Oviposition time was estimated to average 121 seconds (range 13-378) during one series of observations. The numbers of eggs laid on 10 heads in a 2 h period is shown in Table 9. Percent parasitism averaged 35%. Numbers of eggs laid in a 2 h period averaged 12.3/female.

e) Oviposition into larvae - In one experiment, females that were placed on wheat heads for 2 h with exposed first instar midge larvae, (0.429 $\pm$ 0.053 mm long) displayed typical searching behavior but no eggs were found in 35 larvae. In two other similar experiments no eggs were found in 130 first instar midge larvae.

f) Mating - No mating activity was observed during the course of these studies either in the field or laboratory.

5.0 Reproductive potential of P. penetrans

5.1 Females dissected in the laboratory 1986

Females emerged in the laboratory contained the following:

Female No	Mature eggs	Immature eggs	Total
1	142	78	220
2	126	59	185
3	104	51	155
4	23	148	171
5	72	116	186
6	139	0	139
7	192	0	192
Mean (SD)	114 (54)	65 (55)	178 (26)

Table 9. Numbers of eggs laid per female on single heads during a 2 h period in the laboratory at 23°C.

Head Examined	No. midge eggs	No. parasite laid in 2 hrs.	% parasitism	No. parasite eggs laid/min.
1)	68	21	31	0.175
2)	27	13	48	0.108
3)	34	21	62	0.175
4)	24	11	46	0.092
5)	15	3	20	0.025
6)	110	18	16	0.150
7)	34	3	9	0.025
8)	37	1	3	0.008
9)	25	15	60	0.125
10)	24	12	46	0.100
11)	42	21	50	0.175
12)	33	9	27	0.075
Means (SD)	39.4 (25.8)	12.3 (7.3)	34.8 (19.9)	0.103 (0.06)

In 1987, additional dissections (Table 10) shoved that 5 females that had not laid contained about 219 eggs (range 132-268). Most of the females in this experiment had the opportunity to lay only a small compliment of their eggs and so contained large numbers when they died.

5.2 Field-collected females

Females collected in the field July 25, 1985 and dissected in the laboratory contained the following numbers of mature eggs. No immatures were found.

Female No:	1	2	3	4	5	6	7	8	9	10	Mean(SD)
No of eggs:	136	103	123	142	33	122	51	138	109	120	107.7 (37.0)

In the laboratory studies, means for eggs/female were 178 and 219, an average of about 200 eggs for females that had not oviposited. Therefore on the average, the field collected females had oviposited about 53% of their eggs when collected. The fecundity of about 200 eggs per female indicates that under optimum conditions each female could potentially destroy 200 midge eggs.

6.0 Autumn soil survey for wheat midge larvae in cocoons and percent parasitism

In 1986, mean midge density including all 31 RM's was 624 larval cocoons/m^2. The highest midge populations were in A.R.D.'s 12, 18 and 19 (Fig. 24). Midge cocoons were found in 27 of 31 RM's (87%). Percent parasitism varied from 0% in A.R.D. 32 to 87% in A.R.D. 25. Overall RM's averaged 31%.

In 1987 when 52 RM's were sampled, the average midge density was 394/m^2 about 1.6 times less than in 1986. The highest midge densities were again in the A.R.D.'s 12, 18, 19, 42 in eastern Saskatchewan and in 27 (Fig. 25). Midge were found in 43 of 52 RM's (83%). There appeared to be some spread of the midge and the parasite in 1987 since it was found in A.R.D. 33 in 1987 but not in 1986;

Table 10. Number of eggs found in dissected parasite females before and after their oviposition period in the laboratory

Age of female at death (days)	Times female exposed to midge eggs	Total eggs laid	Mature oocytes in abdomen	Oocytes in ovarioles	Total eggs produced
1-3	0	0	219	34	253
	0	0	86	46	132
	0	0	30	173	203
	0	0	46	191	237
	0	0	163	105	268
Mean (SD)			108.8 (80.2)	109.9 (71.5)	218.6 (54.0)
4-7	0	0	116	85	201
	0	0	85	151	236
	1	11	91	34	136
	2	24	118	37	179
	2	50	18	63	131
Mean (SD)		28.3 (19.8)	85.6 (40.5)	74.0 (47.8)	176.6 (44.3)
8-11	1	12	100	126	238
	1	3	124	107	234
	1	21	51	84	156
Mean (SD)		12.0 (9.0)	91.7 (37.2)	105.6 (21.0)	209.3 (46.2)
12-16	0	0	245	23	268
Mean (SD)		0	245	23	268

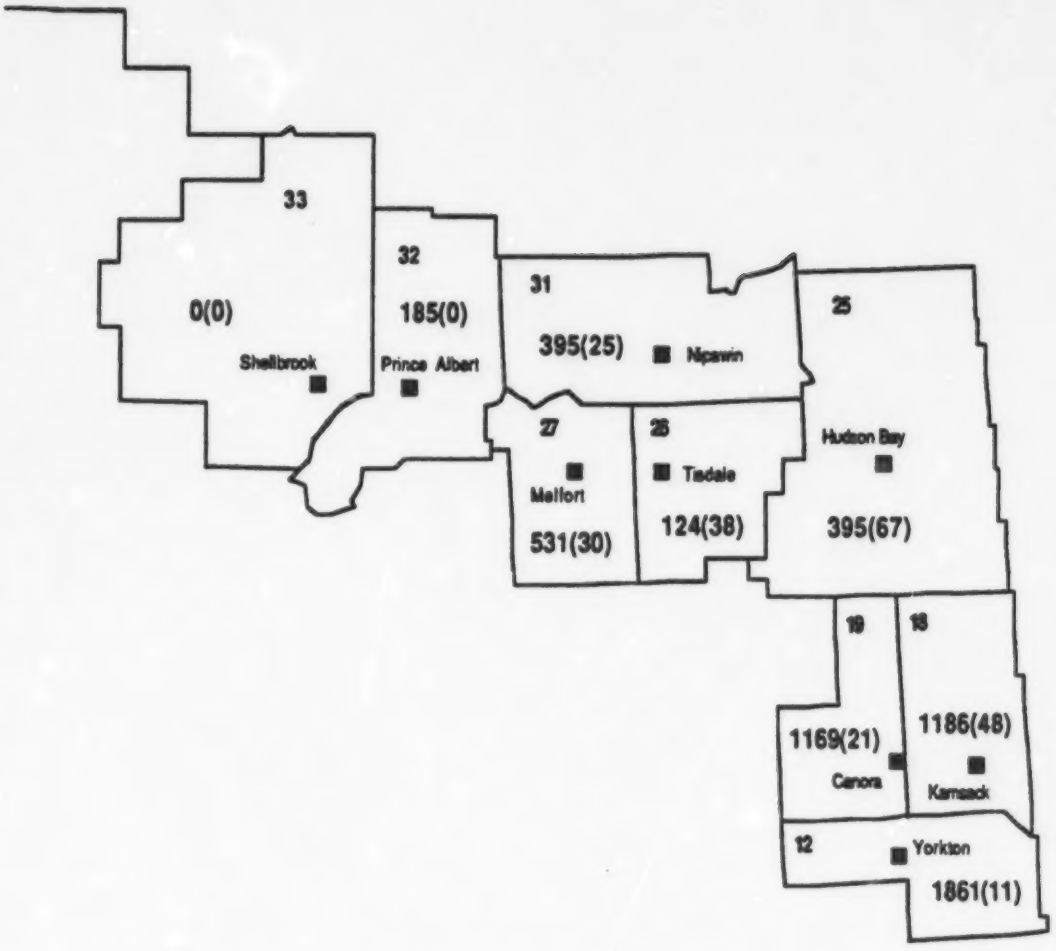

Fig. 24 Mean density of wheat midge cocoons per meter squared in 9 Agricultural Representative districts in 1986, followed by percent parasitism(in brackets) by the wheat midge parasite Pirene penetrans.

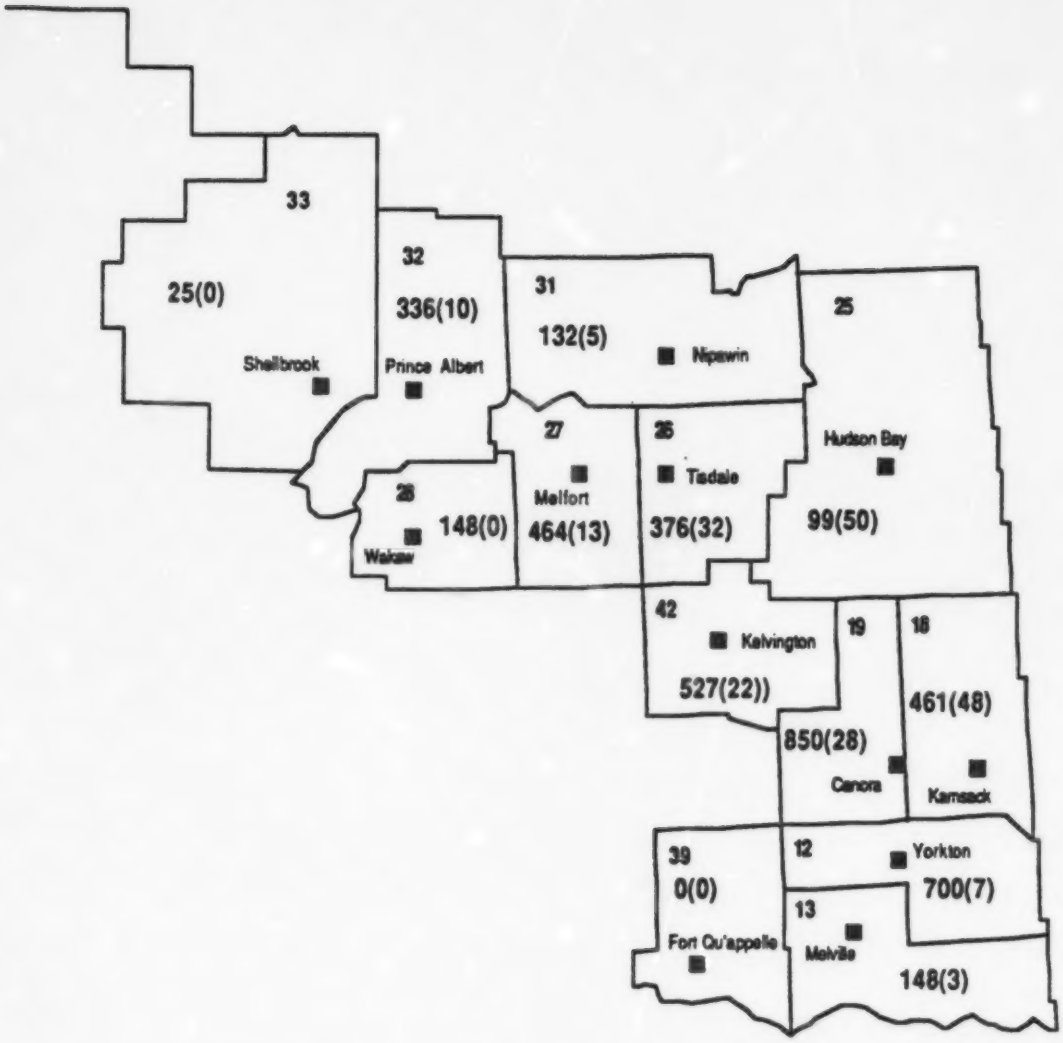

Fig. 25 Mean density of wheat midge cocoons per meter squared in 13 Agricultural Representative districts in 1987, followed by percent parasitism(in brackets) by the wheat midge parasite Pirene penetrans.

while the parasitized larvae were found in A.R.D. 32 in 1987 but not in 1986 (Fig. 24 and 25). Percent parasitism over all RM's averaged 23%.

Regression analysis showed that a density dependent relation existed between parasitized midge larvae and total midge larvae for both years (Fig. 26) when all fields in all RM's were plotted after transformation by $\sqrt{x + 1}$. Regression equations and r^2 values were almost the same for the two years. A density dependent response by a parasite to its host are considered essential attributes for a natural control agent.

Conclusions and Impact

The biology, behavior and impact of a parasite wasp, P. penetrans, of the wheat midge was investigated over a two-year period. An estimated 4000 midge larvae were dissected and examined for parasites during the various phases of the work. No other parasite species other than P. penetrans was found and it is therefore the most important, if not the only parasite, attacking the midge in Saskatchewan.

Morphological studies clearly separated the immature stages and identified characters that could be used to separate P. penetrans from other species that could be introduced in the future. The biology, development period and some of its field behavior are nov known. Environmental conditions could influence levels of parasitism since cloudy, rainy weather could reduce the effectiveness of the parasite.

The soil surveys in autumn showed that there was a direct relationship between the number of parasitized larvae in the soil and the total midge density. This appears to be a density-dependent or delayed density-dependent response of P. penetrans to the density of the host midge. This would imply a "functional response", that is, as midge density increases more midge eggs will be attacked because of their greater availability to the parasites (Hassell 1965). This results in a "numerical response" shown as an increase in numbers of parasites,

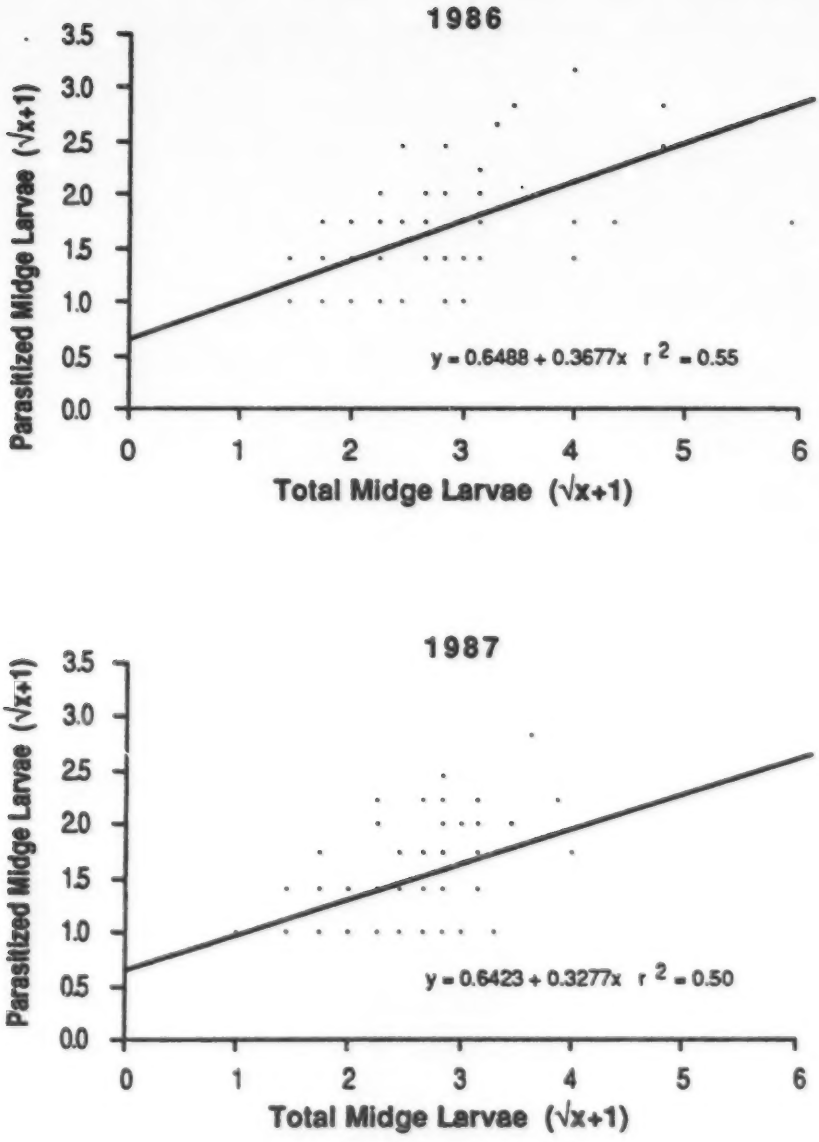

Fig. 26. Relationship between density of midge larvae parasitized by P. penetrans with density of total midge larvae in 124 fields in 31 rural municipalities in 1986 and 208 fields in 52 rural municipalities in 1987.

which is in fact delayed until the following year and seen as a greater emergence of parasites.

The consequence of parasites attacking midge eggs in the wheat heads in 1987 will be a reduction in numbers of midge adults emerging in 1988. As the midge density decreases, as it did from 1986 to 1987 the percent parsitism would also be expected to decrease because of the decrease in availability of prey. In fact, midge density decreased by 37% and percent parasitism by about 8%. The spread and increase of the parasite has appeared to follow the midge by about one year.

A study is underway in Europe to identify other species of parasite that could be introduced into Saskatchevan to supplement the biocontrol given by P. penetrans. Affolter (1987) has found that a complex of four parasites including P. penetrans attack S. mosellana in Europe. The interaction between P. penetrans and the most promising parasites is being studied. The present study to determine the effectiveness of P. penetrans, when it was the only parasite present in Saskatchevan, was necessary before any additional introductions could be considered.

Because of the apparent density-dependent relationship between P. penetrans and the wheat midge, the parasite is considered to be a valuable component of natural control in Saskatchevan. Measures to maintain parasite populations by judicious use of insecticides and adherence to recommended economic thresholds for treatment should be encouraged. Development of cultural or other methods which would lessen the impact of insecticides on parasite populations would be beneficial to midge integrated pest management.

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References

Affolter, F. 1987. The wheat midges Sitodiplosis mosellana and Contarinia tritici. Ann. Rept. C.A.B. Int. Inst. Bio. Control, European Station, 8 pp.

Baker, J.R. 1958. Principles of Biological Microtechnique, Methusen & Co. Ltd. London.

Beirne, B.P. 1955. Collecting, preparing and preserving insects. Pub. 932, Canada Department of Agriculture, 133 pp.

Doane, J.F., O.O. Olfert and M.K. Mukerji. 1987. Extraction precision of sieving and brine flotation for removal of wheat midge, Sitodiplosis mosellana (Diptera:Cecidomijcidae), cocoons and larvae from soil.

Floate, K. 1987. Effect of carabid (Coleoptera:Carabidae) predators on populations of the orange wheat blossom midge, Sitodiplosis mosellana (Gehin) in N. E. Saskatchevan. MS Thesis. Dept. of Biology, University of Saskatchevan.

Hassel, M.P. 1966. Evaluation of parasite or predator responses. J. Anim. Ecol. 35:65-75.

Hinks, C.F. and J.F. Doane. A laboratory method for rearing the orange wheat blossom midge, Sitodiplosis mosellana (Gehin) (Diptera: Cecidomyiidae). J. Econ. Ent. (in press).

Johansson, E. 1936. Studies and research concerning the wheat midge Contarinia tritici Kirby and Clinodiplosis mosellana Geh. and their control. IV. The National Swedish Institute for plant protection 15:1-18 (in Swedish).

Kirby, W. 1800. A continutation of the life history of Tipula tritici, in a letter to Thomas Marsham, Trans Linnean Society 5:96-111.

Kutter, H. 1934. Additional investigations of Kakothrips robustus azel and Contarinia pisi Winn as well as their parasites, particularly Pirene graminea Hal.

Olfert, O.O., M.K. Mukerji, and J.F. Doane. 1985. Relationship between infestation levels and a yield loss caused by wheat midge, Sitodiplosis mosellana (Gehin) (Diptera:Cecidomijiidae), in spring wheat in Saskatchevan. Can. Ent. 117:593-598.